REPERFUSION ARRHYTHMIAS:

mechanisms and prevention

WIEK H. VAN GILST

REPERFUSION ARRHYTHMIAS:
mechanisms and prevention

STELLINGEN

1. Zowel tijdens ischemie als tijdens reperfusie treden ritmestoornissen op die het gevolg zijn van metabole veranderingen in de hartspier; de verschillen tussen beide typen ritmestoornissen worden niet veroorzaakt door de aard van deze veranderingen maar door de snelheid, waarmee zij verlopen.

2. Aan de lijst met klinische situaties, waarin reperfusie een belangrijke rol speelt, kan het fenomeen "myocardial bridging" toegevoegd worden.
(Dit proefschrift).

3. Manning et al. zijn van mening dat allopurinol beschermt tegen reperfusie-aritmieën via een vermindering in de vorming van vrije radicalen; deze bescherming kan echter ook verklaard worden door een effect op het ATP-catabolisme.
(Manning et al., Circ Res 55: 545-548, 1984).

4. Converting enzyme remmers kunnen niet alleen via hemodynamische effekten maar ook via een effekt op het lokale prostaglandine metabolisme ischemische hartziekten gunstig beinvloeden.
(Dit proefschrift)

5. Door farmacologisch onderzoek (autonome blokkade) van de sinusknoop-funktie kan op eenvoudige wijze worden vastgesteld of de stoornis in de sinusknoop zelf, dan wel in de regulatie van de sinusknoop moet worden gezocht.

6. De onenigheid over de prognostische waarde van signal averaging bij het gebruik van anti-aritmica voor ventriculaire tachycardieën zegt meer over de verschillende methoden van kwantificeren dan over het feitelijk belang van late potentials.
(ME Cain et al., Circulation 70, II-252, MB Simson et al., Circulation 72, III-7)

7. Werken met computers valt altijd tegen.

8. De toenemende populariteit van squash verschaft de Nederlandse onderzoeker een model voor plotselinge hartdood, waarbij reperfusie een belangrijke rol speelt.

9. Het arbeidstijdverkorting-beleid van de universiteit, dat wordt gekenmerkt door het toekennen van roostervrije dagen zonder herbezetting van vrijgekomen plaatsen, werkt veeleer een kwaliteits vermindering van onderzoek en onderwijs in de hand dan dat het een bijdrage levert aan een daling van de werkeloosheid onder jonge academici en dient daarom als een arbeidstijdverspilling-beleid te worden beschouwd.

10. De vele schooltypen voor kinderen met leer- en/of opvoedingsproblemen zijn algemeen aanvaard. De mogelijkheid direct na het basisonderwijs naar het zelfstandig gymnasium te gaan, verdient eenzelfde acceptatie.

11. Het Zuid-Afrikaanse argument: "Je moet er geweest zijn om er over te kunnen oordelen", is niet volledig, aangezien het voorbijgaat aan het feit dat het bedoelde beoordelingsvermogen een bifasisch verloop kent en blijkbaar afneemt bij langer verblijf.

Stellingen behorende bij het proefschrift van W.H. van Gilst, "Reperfusion arrhythmias: mechanisms and prevention." Groningen, 19 maart 1986

RIJKSUNIVERSITEIT TE GRONINGEN

REPERFUSION ARRHYTHMIAS:
mechanisms and prevention

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op woensdag 19 maart 1986
des namiddags te 4.00 uur
door

WIEKERT HENDRIKUS VAN GILST

geboren te Pretoria

1986
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotores : Prof. Dr. H. Wesseling
Prof. Dr. K. I. Lie
Prof. Dr. W. G. Nayler

Referenten : Dr. C. D. J. de Langen
Dr. J. H. Kingma

Financial support by: Squibb B.V.,
Lorex Pharmaceutica B.V.,
Schering Nederland B.V.,
Hoechst Holland N.V.
for the publication of this thesis is gratefully acknowledged.

ISBN 90 367 0012 4

aan mijn ouders

voor Trix

CONTENTS

CHAPTER I

GENERAL INTRODUCTION AND AIMS OF THE THESIS

As was already noted over 4000 years ago, a man who simultaneously espouses two opposing view points should be viewed with suspicion. Yet this thesis will take that risk by arguing that restoration of coronary blood flow in the ischemic myocardium is absolutely needed to prevent irreversible cellular damage but should be feared for its potentially hazardous consequences. This paradox is illustrated by recent progress in methods that improve myocardial oxygen supply to the acutely ischemic myocardium and thus reduce cellular damage (Rentrop et al. 1981; Mathey et al. 1981; Markis et al., 1981) on the one hand and the early demonstration by Tennant and Wiggers that providing such supply by reperfusion of the ischemic myocardium is associated with malignant ventricular arrhythmias (Tennant and Wiggers, 1935). Though the latter finding dates from 50 years ago, elucidation of reperfusion phenomena has only recently taken on a new significance because of the above mentioned clinical developments and the revival of the concept of coronary spasm in the setting of ischemic heart disease (see also Chapter V).

The firm establishment of the concept of coronary artery spasm (Oliva and Breckenridge, 1977) has led to the suggestion that reperfusion induced ventricular fibrillation may be a major cause of

sudden cardiac death in man (Elharrar and Zipes, 1977). In the majority of sudden death victims, the final common pathway involves a malignant ventricular arrhythmia. The recent report of the Aspirin Myocardial Infarction Study estimated that 97.5% of patients dying within 1 hour of the onset of symptoms had a lethal arrhythmia as a cause (Goldstein et al., 1984). Although increased ventricular vulnerability to fibrillation may occur secondary to complete coronary occlusion, no complete coronary occlusion could be demonstrated in a large percentage of patients autopsied after sudden cardiac death (Bashe et al., 1975; Baba et al., 1975; Schwartz and Gerrity, 1975; Davies and Thomas, 1984). In the light of the evidence presented above, the lethal event may have been due to reperfusion after an ischemic episode. If so, one or more of the following mechanisms may be responsible for this phenomenon:

1) The already mentioned spasm of a normal or partially obstructed coronary artery and its subsequent relaxation (Tzivoni et al., 1983; Corr and Witkowski, 1983; Oliva and Breckenridge, 1977; Kerin et al., 1983).

2) Platelet aggregation with thrombus formation and subsequent spontaneous lysis (Jorgensen et al., 1967).

3) Increase in collateral flow to an ischemic region (Marcus et al., 1976).

Apart from these spontaneously occurring sequences of events, reperfusion is also encountered after recanalization of a previously occluded coronary artery by angioplastic or thrombolytic procedures in the setting of acute myocardial ischemia or infarction (Rentrop et al., 1981; Mathey et al., 1981; Markis et al., 1981; Goldberg et al., 1983).

Although the incidence of severe arrhythmias appears to occur with variable frequency in these patients, recent studies indicate that the presence of ventricular arrhythmias during intracoronary thrombolysis is an indicator of the success of recanalization (Goldberg et al., 1983; Corr and Witkowski, 1983).

Furthermore, reperfusion of the whole heart occurs under the surgical conditions of cardiopulmonary bypass with ischemic cardiac arrest. During surgical reperfusion, various serious arrhythmias, including ventricular fibrillation, are frequently encountered (Manning and Hearse, 1984).

In this thesis reperfusion arrhythmias are defined as any disturbance of cardiac rhythm that arises as a consequence of the total or partial restoration of perfusion in myocardial tissue following global or regional ischemia.

The mechanisms involved in occlusion and reperfusion arrhythmias appear to differ (Axelrod et al., 1975; Levites et al., 1975; Corbalan et al., 1976; Penkoske et al., 1978; Zuanetti et al., 1985). The malignant arrhythmias associated with coronary occlusion, including both ventricular tachycardia and ventricular fibrillation, are usually preceded by gradual alterations in the electrical behaviour of the ischemic and border regions. In contrast, reperfusion arrhythmias usually occur within seconds, persist for a brief period, and frequently commence with the occurrence of ventricular fibrillation (van Gilst and de Langen, 1982;van Gilst et al., 1984; Penkoske et al., 1978; Kaplinsky et al., 1981). Intracellular electrode recordings revealed that ischemia results in a time-dependent progressive, heterogeneous decrease in

action potential duration and amplitude, resting membrane potential and maximum rate of rise of voltage of phase 0 (Downar et al., 1977, Russell et al., 1977; Russell and Oliver, 1978. Janse and Kleber, 1981). Reperfusion with arterial blood results in a rapid return of electrical activity to the ischemic zone (Corr and Witkowski, 1984). Restoration of the amplitude of the action potential in the ischemic zone occurs within a few cardiac cycles (Penkoske et al., 1978; Kaplinsky et al., 1981; Downar et al., 1977) whereas the desynchronization of electrical depolarization, reflected by widening or fractionation of the electrograms, requires minutes to recover (Penkoske et al., 1978; Kaplinsky et al., 1981). Although some reports indicate a prolongation in refractory period and action potential duration (Levites et al., 1975; Stewart et al., 1980), a further shortening of the effective refractory period and action potential during the first seconds of reperfusion has been found by many other investigators (Penkoske et al., 1978; Penny and Sheridan, 1983; Naimi et al., 1977). This latter finding indicates an increased dispersion of refractoriness between the ischemic and non-ischemic myocardium, which adds to already existing electrical inhomogeneity of the heart. This reperfusion induced heterogeneity of cellular injury and recovery may provide the necessary electrophysiological conditions for the genesis of arrhythmias (Ferrier et al., 1985). On the basis of studies in the pig, Janse (Janse and Kleber, 1981) has suggested that ventricular tachycardia and fibrillation may arise as a consequence of the sudden return of electrical activity to previously inexcitable tissue and the inhomogeneity of this process would support reentry mechanisms. The

concept of reentry mechanisms as an important factor in the genesis of reperfusion arrhythmias has been supported by several investigators using different models (Levites et al., 1975, Murdock et al., 1980; Fujimoto et al., 1983). Recent approaches using epicardial mapping procedures have shown that the earliest evidence of epicardial breakthrough which initiated ventricular fibrillation after reperfusion was from the "border zone" of the ischemic region (Ideker et al., 1981). This confirms the role of heterogeneity in the genesis of reperfusion arrhythmias. Fujimoto et al. (1983) concluded from studies in canine hearts that in a vast majority the site of initiation of reperfusion arrhythmias was situated in the "border zone". However, the maintenance of this arrhythmia and its deterioration into ventricular fibrillation involved reentry, utilizing the maximally depressed cells more to the center of the previously ischemic zone.

Reports on the role of enhanced automaticity in the development of reperfusion arrhythmias have been conflicting. Several investigators failed to demonstrate enhanced automaticity (Murdock et al., 1980; Levites et al., 1975). However, Penkoske et al. (1978) demonstrated an accelerated idioventricular rate in vagally arrested cat hearts reperfused after 35 min of ischemia. Furthermore, they noted that rapid atrial pacing suppressed the reperfusion arrhythmias and they concluded that enhanced automaticity may play a role in the initiation of reperfusion arrhythmias. Recently, it has been suggested that an increase in idioventricular rate associated with reperfusion may be mediated by alpha-adrenergic mechanisms (Sheridan et al., 1980; Corr and Witkowski, 1984). This may also explain the commonly found accelerated

idioventricular rhythms in patients undergoing intracoronary thrombolysis, particularly if the site of occlusion is in the anterior descending branch of the left coronary artery (Goldberg et al., 1983). On the basis of these conflicting data, Manning and Hearse (1984) suggested that similar to ischemia induced arrhythmias, reperfusion arrhythmias may be divided into distinct categories arising at different times and occurring as a result of different mechanisms. Evidence for this proposition was derived from a study by Kaplinsky et al., who demonstrated two distinct types of reperfusion arrhythmias (Kaplinsky et al., 1981). One type occurring during the first 60 sec of reperfusion with a high incidence of ventricular fibrillation was proposed to be due to reentry. The second type occurred between 2 and 7 min after the onset of reperfusion and had a significantly lower incidence of ventricular fibrillation. This type was thought to be due to enhanced automaticity. Ferrier and colleagues observed during this phase in isolated canine ventricular tissue enhanced automaticity at low membrane potentials, during which Purkinje tissue functioned as a parasystolic focus (Ferrier et al. 1985).

In this thesis the following four main avenues have been investigated:

1. Both in vitro and in vivo models have been developed to study the potentially causal electrophysiologic and biochemical mechanisms which underlie reperfusion-induced mechanisms (Chapter II).

2. In these models factors which modulate reperfusion-induced arrhythmias have been studied (Chapter III).

3. Several drugs have been shown to prevent or delay reperfusion phenomena. Their tentative modes of action led to studies with calcium-antagonists, prostacyclin analogues and converting-enzyme inhibitors (Chapter IV).

4. The working hypothesis on reperfusion arrhythmias, based on the experimental data, was tested in the clinical situation (Chapter V).

The majority of our experiments have resulted in papers published elsewhere. These publications are added to this thesis in the appendix. In the following chapters these results are discussed and placed in a more general context.

CHAPTER II

EXPERIMENTAL MODELS

Reperfusion arrhythmias and their treatment are expanding areas of research. None of the models suffices for all sequelae, thus a variety of experimental models is needed to cover all aspects of the pharmacology, pathology and biochemistry related to these arrhythmias. For this reason in our studies we have used different models of different species, both in vivo and in vitro. These range from an isolated rat heart to an in vivo monkey preparation, each of which has its own inherent advantages and disadvantages, which will be discussed in this chapter.

A. The isolated rat heart

Since 1895, the Langendorff preparation in which the isolated heart is perfused retrogradely through the aorta under constant pressure or constant flow (Langendorff, 1895) has been used extensively. Hearts from a variety of small mammals (cat, mouse, guinea-pig, rabbit, rat) have been used for perfusion although the rabbit and particularly the rat heart have been used most often. They offer the advantages of low cost and ease of availability. They can be studied rapidly in large numbers

and results are highly reproducible. Experimental conditions can be very strictly controlled and a great many of parameters measured (Manning et al., 1980; de Leiris et al., 1984; van Gilst et al., 1984a). However, there are clear and obvious differences between the isolated rat heart and the heart of patients with severely diseased coronary vessels. Such differences include heart size and rate, age of the myocardial tissue, pre-existing coronary collateral vessels, and the site (global vs regional) and timing (sudden vs gradual) of the occlusion and subsequent reperfusion.

We have already pointed out in chapter I that reperfusion arrhythmias are probably due to reentry (at least the early arrhythmias). The conditions necessary for reentry are based on the heterogeneity of recovery processes added to the pre-existing heterogeneity of ischemic injury of the myocardium. Therefore, it can be envisaged that the use of regional ischemia in the isolated rat heart model is more suitable to mimic this situation of heterogeneity than global ischemia. In a comparison of these two situations we found that 15 min regional ischemia resulted in a 100% incidence of ventricular fibrillation upon reperfusion, while globally ischemic hearts showed no ventricular fibrillation upon reperfusion after 15 min ischemia (van Gilst and de Langen, 1982). Based on these results we used in our further experiments in the isolated rat heart regional, reversible ischemia according to the method of Kannengieser (Kannengieser et al., 1975) which model is also validated by others (Lubbe et al., 1978). A detailed description of this model and the perfusion apparatus can be found in appendices IV and VI.

B. Coronary occlusion and reperfusion in the in vivo rat heart

In 1946 Heimburger first used the in vivo rat as a model for myocardial infarction (Heimburger, 1946). The animal was ventilated and the ligation was performed through a thoracotomy window. A similar technique was used by Johns and Olson to investigate the effects of left coronary artery ligation on macro- ar.d microscopic structures of the heart (Johns and Olson, 1946). In 1960, Selye published a rapid procedure for ligating the left coronary artery in which the rats were not ventilated (Selye et al., 1960). Nevertheless, the technique was little used thereafter until recently (Kloner et al., 1977; MacLean et al., 1978). In vivo rat preparations have since been used to demonstrate antiarrhythmic and cardioprotective actions of a great variety of drugs (Marshall et al., 1981; Kane et al., 1982; Johston et al., 1983; Evans et al., 1985). However, in all these studies permanent occlusion of the left coronary artery was used. Only in a few studies reperfusion of the ischemic zone in the rat heart in vivo is performed (Bergey et al., 1982; Manning and Hearse 1984). Since we had decided that regional and temporarily obstruction of myocardial flow gave satisfactory results in the in vitro model, we wished to follow the same protocol in the in vivo situation, i.e. the anesthetised rat.

C. A closed-chest model of reperfusion-induced arrhythmias in the pig

This large in vivo preparation was chosen because ample evidence has been presented that the pig coronary vascular system is more closely related to that of man than the dog system (Eckstein, 1954; Schaper, 1979; White and Bloor, 1981). In pigs, the right coronary and left anterior descending artery are about equal in size, each supplying about 40% of the porcine myocardium with blood. In this species the left circumflex artery plays a minor role (Verdouw et al. 1983). Postmortem studies of the porcine heart also demonstrated similarities with the human circulation as far as intramural branching patterns, supply to papillary muscles and nodal conduction tissue are concerned (Eckstein, 1954; Brooks et al., 1975).

Probably the most important reason for preferring the pig model is that the pig heart, in contrast to the dog, lacks significant collateral blood flow (White and Bloor, 1981; Schaper et al., 1967; Lumb and Singletary, 1962). A nearly complete absence of collateral flow is found in two-thirds of patients with acute myocardial infarction (Rousseau et al., 1982). Furthermore, this lack of significant collateral blood flow facilitates the comparison between control and treated hearts (Klein et al., 1984a), which is known to be somewhat difficult in canine preparations (Lowe et al., 1978). Also the spatial and temporal evolution of ischemic injury in pig hearts (Klein et al., 1984b) differs from that in dog hearts (Schaper et al., 1979). As already pointed out in chapter I this development of injury may be one of the most important factors leading to reperfusion arrhythmias.

A detailed description of the applied methods can be found in appendix VI.

D. Coronary occlusion and reperfusion in the monkey heart

Because primates are phylogenetically close to man, one can assume that creation of myocardial ischemia with subsequent reperfusion in these animals will provide an ideal model of events in man. This is especially so for work involving immunological processes and the molecular structure of receptors. This last point may be of importance for validating the concept that during ischemia, alpha-1 receptors increase in the ischemic tissue (Sheridan et al., 1980; Corr et al., 1981). Furthermore, creatine kinase activity, especially the MB creatine kinase isoenzyme of the myocardium, appears to be more closely related to that in man, as has been shown in the baboon (Yasmineh et al., 1976). This similarity makes the study of creatine kinase kinetics during ischemia and reperfusion more meaningful. Such studies can be used in the clinical situation as a marker of spontaneous reperfusion in acute myocardial infarction or successful recanalization (Ong et al., 1983).

In primates, at least in the baboon (Opie et al., 1975; Opie et al., 1983), coronary artery occlusion results in a very well circumscribed lesion, with a clearly defined edge and with marked tissue metabolic changes, since collateral blood flow to the infarct zone is low. The degree and patterns of ST segment changes appear to be very similar to those in pigs (Smith et al., 1979).

However, despite all these advantages, extensive use of this particularly suitable model is not justified for economical and ethical reasons. Nevertheless, the potential implications of the experimental data reported in this thesis have warranted the need to validate our reperfusion protocol in a model closer to the human situation. Accordingly, we have analyzed the relationship between 15 min coronary artery occlusion with subsequent reperfusion and the incidence of reperfusion arrhythmias in anesthetized monkeys (van Gilst et al., 1984b). Furthermore, creatine kinase values were monitored during the experiment. These experiments confirmed the high incidence of ventricular tachycardia and ventricular fibrillation upon reperfusion after relative short periods of ischemia also seen in our other experimental models. The results also indicate the value of plasma creatine kinase - kinetics for the assessment of spontaneous reperfusion in patients (see also Ong et al., 1983).

CHAPTER III

CELLULAR EVENTS MODULATING REPERFUSION ARRHYTHMIAS

Ischemia preceding reperfusion is a dynamic process involving a wide range of cellular events, such as loss of ionic homeostasis, ultrastructural damage, depletion of ATP and creatine kinase, increase in cyclic AMP, increase in lysophosphoglycerides and long-chain acyl carnitines, increase in alpha adrenoreceptors, prostaglandin release, catecholamine release, loss of intracellular components, increase in myocardial lactate, etc, etc. All these events together will set the stage for the occurrence of arrhythmias upon reperfusion and, since their progression is dependent on both duration and severity of ischemia, it is also conceivable that the extent of reperfusion arrhythmias is related to these two factors. From our studies in the isolated rat heart there appears to be an inverse relationship between the duration of ischemia and the occurrence of reperfusion arrhythmias, such that after 15 min ischemia, almost 100% of the hearts exhibit ventricular fibrillation upon reperfusion (Van Gilst et al., 1984c; appendix III), but almost none after 60 min ischemia (van Gilst et al., 1984c). If studied in detail, the curve representing duration of ischemia versus incidence of ventricular fibrillation appears to be bell shaped. This was also found in the isolated rat heart by Crome et al. (1983) and in the isolated guinea-pig heart by Penny and Sheridan

(1983). A comparable relationship is found in our closed-chest pig model (de Graeff et al., 1986) and in the dog (Balke et al., 1981). It has been suggested that the decline in vulnerability to ventricular fibrillation is due to the onset of irreversible cellular injury (Manning and Hearse, 1984) and that the vulnerability is maximal when a maximal number of cells in the ischemic zone is in the late phase of reversible injury (Hearse, 1983). Apparently, either cell death or no injury at all will lead to no electrical activity or normal electrical function respectively, whereas marked heterogeneity of cell function in the late phase of reversible injury will lead to heterogenous electrical activity facilitating reentry.

In this chapter several of the cellular changes which occur at this stage of events and their role in arrhythmogenesis upon reperfusion will be discussed individually.

A. Calcium and reperfusion arrhythmias

Myocardial cells in the ischemic zone will accumulate calcium from the extracellular space. However, during total ischemia the available amount will be limited and the concentration gradient will decrease, which results in a relatively small net gain in cellular calcium during total ischemia (Nayler et al., 1980b; Shen and Jennings, 1972). Upon reperfusion an abundant amount of calcium is again available and the driving force of the concentration gradient will be reinstituted.

The concentration of tissue calcium may increase up to 8 to 10 times over control during the early phase of reperfusion (Whalen et al., 1974; Hearse, 1977; Shen and Jennings, 1972; Jennings and Reimer, 1981; Nayler, 1981; Bourdillon and Poole-Wilson, 1981). This occurs under conditions in which there is incomplete recovery of mechanical function (irreversible injury), and ultra structural evidence of myocardial injury aggravated by reperfusion (reperfusion damage).

The actual calcium accumulated is far more than can be accounted for by edema alone and is due chiefly to the active accumulation of calcium in mitochondria in the form of characteristic granular densities of calcium phosphate (Shen and Jennings 1972).

Several authors have discussed this process of calcium-overloading as a potential cause of cell death or cellular dysfunction (Nayler, 1981; Farber, 1981; Bourdillon and Poole Wilson, 1981; Jennings, 1984; Katz and Reuter, 1979). However, the pathophysiological mechanisms underlying this reperfusion induced calcium uptake are still unknown. Suggested mechanisms include slow channel dysfunction (Nayler et al., 1985), influx of calcium in exchange for sodium (Bourdillon and Poole-Wilson, 1981; Grinwald, 1982), peroxidation of membrane lipids (Hess et al., 1981b; Guarnieri et al., 1980; Gauduel and Duvelleroy, 1984; see also section D of this chapter), excessive calcium release from intracellular stores (Dunnett and Nayler, 1979), adrenergic receptor stimulation (Sharma et al., 1983) and lysophosphoglycerides (Sedlis et al., 1983).

The total calcium carrying capacity of normal functioning slow channels is probably insufficient to account for the rapid and massive

influx of calcium during reperfusion (Krishtal et al., 1981; Kostyuk et al., 1981). However, it has been suggested that due to a change in phospholipid metabolism, which is known to play a role in regulating slow channels (Putney et al., 1980) the calcium carrying activity of the slow channels can increase (Nayler, 1985). The increased amount of phospholipids may also form specific ion channels in cell membranes (Lee and Chang, 1977). Recently, Sedlis et al. reported a reversible increase in calcium uptake in cultured myocardial cells in response to lysophosphatidylcholine (Sedlis et al., 1983).

Another possible route of calcium entry may be the calcium/sodium exchange mechanism (Grinwald, 1982). Due to ATP-depletion during the ischemic period the Na/K-activated ATPase enzyme may be impaired resulting in sodium accumulation which could then be exchanged for calcium (Reuter, 1974), causing an early influx of calcium into the tissue. However, recent findings indicate that the rate of this process is too slow to account for all of the calcium that enters during postischemic reperfusion (Nayler et al., 1985).

Entry through alpha adrenergic receptor controlled pathways could be involved, particularly as there is an increase in numbers of alpha adrenergic receptors in ischemic myocardium (Corr et al., 1981). Sharma reported that alpha-blockade inhibited calcium gain in reperfused ischemic myocardium (Sharma et al., 1983). However, calcium uptake studies in reoxygenation experiments performed in the isolated intraventricular septum preparation showed no key role for alpha-adrenergic receptor stimulation (Tones and Poole-Wilson, 1985).

A field of great interest at present is the role of oxygen derived free radicals during reperfusion. Peroxidation of membrane lipids (Guarnieri et al., 1980) may influence calcium entry and cellular calcium distribution (see also section D).

Thus, although a gain in cytosolic calcium upon reperfusion seems fairly established, the mechanism leading to this calcium overload is far from elucidated and probably a multiplicity of mechanisms is involved. The electrophysiological consequences of this uncontrolled calcium entry is also largely a matter of debate. It has been suggested that an elevated intracellular calcium concentration in depolarized tissue, which is prominent during ischemia and the early stage of reperfusion, may result in oscillatory activity that could reach threshold and produce multiple ectopic depolarizations (Corr and Witkowski, 1984). However, since ischemia leads to a complex alteration in the myocardial cell as mentioned previously, it is difficult to conclude from experimental ischemia-reperfusion models whether reperfusion arrhythmias are exclusively triggered by intracellular calcium overloading or that other factors are possibly involved in this pathogenetic process. In order to study the role of calcium overloading in the pathogenesis of arrhythmias we used the "calcium paradox" model (Zimmerman and Hulsmann, 1967). Many investigators have shown that this is a useful model to study the effects of calcium overload. A clear relationship was found between the duration of calcium-free perfusion and the incidence and duration of subsequently induced calcium-repletion arrhythmias (van Gilst and Koomen, 1985; Appendix V). Although this relationship makes the conclusion that calcium overloading plays a role

in arrhythmogenesis most attractive, it must be borne in mind that we are dealing here with a model which only partly mimics the ischemia-reperfusion situation. For instance, there is no accumulation of potentially toxic metabolites such as lysophospholipids, potassium, catecholamines and free fatty acids in the calcium-paradox model.

Furthermore, we studied the effects of magnesium on reperfusion arrhythmias, since magnesium has been shown to counteract the actions of calcium in many situations (Iseri and French, 1984). Of importance is the observation that low magnesium concentrations potentiated and high magnesium concentrations blocked the afterdepolarizations caused by strophantidin (Kass et al., 1978). Ferrier further indicated that these oscillatory afterpotentials are promoted by low intracellular potassium and high calcium levels (Ferrier, 1977) which may represent the situation found in the early phase of reperfusion. In our study magnesium was able to reduce the incidence of reperfusion arrhythmias without altering myocardial injury (van Gilst et al., 1984c).

B. Role of prostaglandins

Prostaglandins appear in increasing amounts in coronary venous blood when a major coronary artery is occluded and when the occlusion is subsequently released (Alexander et al., 1975, Kraemer et al., 1976; Ogletree et al., 1977; Berger et al., 1977). There is a significant increase in tromboxane A2 and prostacyclin concentrations. The release and reperfusion induced washout of thromboxane A2 can be related to the

occurrence of cardiac arrhythmias (Coker et al., 1981a; Coker et al., 1982a; Coker, 1984b). Furthermore, it appears that early arrhythmias are related to the balance between thromboxane and prostacyclin release. Preponderance of thromboxane release is associated with the occurrence of more arrhythmias whereas fewer arrhythmias occur when the balance is in favour of prostacyclin release (Coker et al., 1981b). All these studies show only indirectly that thromboxane A2 and prostacyclin are involved in ischemia-reperfusion phenomena. Whether these substances posses direct arrhythmogenic or antiarrhythmic properties is not known at present. There are a number of possible indirect mechanisms which may explain the effects of thromboxane and prostacycline.

Thromboxane A2 is a potent stimulator of platelet aggregation (Hamberg et al., 1975) and coronary vascular smooth muscle contraction (Ellis et al., 1976). Prostacyclin is the physiological antagonist of thromboxane (Lefer et al., 1978). Cyclic local coronary imbalances of these substances may form the basis of reperfusion phenomena via fluctuating spasms or platelet aggregation and subsequent resolution (Aiken et al., 1979). Abnormal coronary prostaglandin levels have been found in patient with unstable angina, variant angina or acute myocardial infarction, either during or following an ischemic event (Serneri et al., 1985; Friedrich et al., 1985; Walinsky et al., 1984; Hirsh et al., 1981; Lewy et al., 1979; Robertson et al., 1981; Tada et al., 1981).

Another possible explanation of the indirect role of thromboxane and prostacyclin in reperfusion-induced arrhythmogenesis is their effect on ischemia induced cellular injury produced by coronary artery

occlusion. Infusion of exogenous prostacyclin has been shown to favourably modify cellular injury evoked by a regional or global reduction in myocardial blood flow. After coronary artery ligation, reduced enzyme depletion and a stabilization of myocardial lysosomes in ischemic tissue have been observed both in the anesthetized cat in vivo (Ogletree et al., 1979) and in the isolated cat heart (Araki and Lefer, 1980). Furthermore, a decrease in the extent of myocardial necrosis has been shown to occur in conscious (Jugdutt et al., 1981) and anesthetized dogs (Melin and Becker, 1983). A protective effect of prostacyclin in the absence of platelets has also been shown in the ischemic and reperfused isolated rat heart (Nayler et al., 1984). Despite these observations the precise mechanism which is responsible for the protective effect of prostacyclin is still unknown. Recent studies indicate that prostacyclin preserves intraneuronal catecholamines in adrenergic nerves in the ischemic myocardium, presumably by inhibition of catecholamine release (Schrör et al., 1982; Schrör and Funke, 1985). Since locally released noradrenaline is probably involved in the genesis of severe ventricular arrhythmias in myocardial reperfusion (see also section C), an imbalance in the thromboxane and prostacyclin ratio influencing local noradrenaline release may thus affect reperfusion arrhythmias.

C. Catecholamines and reperfusion arrhythmias

The importance of the sympathetic nervous system in modulating the course of myocardial ischemia has been demonstrated both in clinical (Peter et al., 1978; Yusuf et al., 1980; Hjalmarson et al., 1981; Karlsberg et al., 1981) and experimental studies (Corr and Gillis, 1978; Hjalmarson, 1980; Maroko et al., 1971; Schaal et al., 1969). This modulation has been shown to contribute to metabolic derangements resulting in myocardial cell damage (Reimer et al., 1976) and severe rhythm disturbances (Ebert et al., 1970; Menken et al., 1979; Schwartz and Stone, 1980; Opie et al., 1979). It is postulated that as a consequence of catecholamine stimulation cyclic AMP tissue levels increase and may provoke these ischemic cardiac arrhythmias (Podzuweit et al., 1978; Opie, 1980). Cyclic AMP enhances the entry of calcium through the slow channels (Sperelakis and Schneider, 1976; Rinaldi et al., 1982) and this could precipitate a variety of electrophysiological disorders, including slow responses, electrical uncoupling and afterdepolarizations.

Reperfusion of the ischemic myocardium has been shown to result in a suddenly increased output of noradrenaline in the coronary effluent (Abrahamsson et al., 1983; Didier et al., 1980; Van Gilst et al., 1985a; Schömig et al., 1984). This enhanced overflow of noradrenaline may be induced by the reperfusion per se or, more likely, noradrenaline which is lost from the adrenergic neurons during the ischemic period may accumulate in the ischemic area and thus, together with formed metabolites be washed out during reperfusion. When ischemic isolated rat

hearts were reperfused with a calcium-free medium, no reduction in noradrenaline overflow was observed (Abrahamsson et al., 1984). It was concluded from this study that noradrenaline is released during ischemia and washed out at reperfusion, since a major part of the acute cellular damage produced at reperfusion is calcium-dependent. However, other studies show that postischemic low calcium perfusion has almost no protective effect on myocardial injury (Koomen et al., 1983). Thus, the mechanism underlying reperfusion-induced catecholamine release is still unclear at present.

This release from endogenous myocardial catecholamine stores has been reported to correlate with the incidence of reperfusion arrhythmias (Corr and Gillis, 1978). In experimental animals, chronic cardiac neural ablation significantly reduces the incidence of ventricular fibrillation upon reperfusion, whereas acute cardiac denervation has little effect (Ebert et al., 1970). Also chemical depletion of myocardial catecholamines using 6-hydroxydopamine is highly effective in preventing ventricular tachycardia and fibrillation associated with myocardial reperfusion (Sheridan et al., 1980). Pretreatment with reserpine (2 mg/300 g) resulted in our isolated rat heart model in a significant reduction of ventricular fibrillation upon reperfusion. This catecholamine depletion has been shown to abolish or attenuate reperfusion induced shortening of action potential duration and refractory period (Penny, 1984; Culling et al., 1984). These electrophysiological changes contribute to the arrhythmias associated with reperfusion (Chapter I).

Several investigators have reported that beta-adrenergic receptor blockade fails to alter the malignant arrhthmias associated with reperfusion (Sheridan et al., 1980; Sugiyama et al., 1980b; Coker and Parratt, 1984) or the sudden reduction in the ventricular fibrillation threshold that follows immediately after the release of a coronary occlusion (Corbalan et al., 1976). In studies which showed a reduction in reperfusion arrhythmias following the administration of beta-adrenoceptor antagonists, the beta-antagonist activity of the compounds tested could not account for the inhibition of reperfusion ventricular arrhythmias (Thandroyen et al., 1983; Rochette et al., 1984). Probably the observed effects were due to membrane-stabilizing activity at high concentrations.

Recent studies have shown the efficacy of alpha-adrenergic receptor blocking drugs in reducing the incidence of arrhythmias during reperfusion (Thandroyen et al., 1983; Corr and Crafford, 1981; Davey, 1980). Both enhanced alpha-adrenergic responsiveness (Sheridan et al., 1980) and a nearly twofold reversible increase in the number of alpha-1 adrenergic receptors (Corr et al., 1981) have been described. This increase in the number of alpha-1 adrenergic receptors persists during early reperfusion but is no longer evident after 15 min of reperfusion (Corr et al., 1981), a time course identical to the enhanced alpha-adrenergic responsivity (Sheridan et al., 1980). As already pointed out in chapter I, alpha-receptor stimulation may increase the slow inward calcium current (Corr and Witkowski, 1984) and could result in multiple ectopic depolarizations. However, comparable to studies with beta adrenoceptor antagonists, recent studies have suggested that the most

appropriate explanation for the antiarrhythmic effects of alpha-antagonist agents is a "membrane-stabilizing" effect (Thandroyen et al., 1983; Bralet et al., 1985).

In conclusion, an involvement of catecholamines in the generation of arrhythmias upon reperfusion seems fairly well established. However, the precise role of alpha or beta adrenergic receptors as transducers of the enhanced catecholamine overflow is not fully understood at present. Possibly other and totally different mechanisms are involved such as the production of free radicals due to degradation of accumulated catecholamines (See also section D)

D. Role of free radicals

A field of great interest at present is the role of oxygen derived free radical species in ischemia and reperfusion. Under normal conditions free radicals, mainly superoxide radicals, are formed in mitochondria during respiration. The damaging effects of these reactive species are due to their ability to oxidise nucleic acids, unsaturated lipids , and proteins. The protection from superoxide toxicity in heart cells is provided by superoxide dismutase, which catalyzes the dismutation of superoxide to hydrogen peroxide in cytoplasma and mitochondria. Because of the low catalase activity in heart cells, the further degradation of hydrogenperoxide to water is catalyzed mainly by glutathione-peroxidase, using glutathione as a hydrogen donor. This leads to interconversion of glutathione into its oxidized form. During

ischemia a decrease in these cellular defense mechanisms against free radicals in the heart has been shown (Julicher et al., 1984). Several authors have suggested that the pathogenesis of cardiac ischemic injury and the exacerbation by reperfusion can be ascribed to the production of free radicals (Guarnieri et al., 1980; Hess et al., 1981a; Lefer et al., 1981; Shlafer et al., 1982; Gauduel and Duvelleroy, 1984; Rao and Mueller, 1983) and subsequent lipid peroxidation (Meerson and Ustinova, 1982).

The major source of superoxide in postischemic tissues appears to be the enzyme xanthine oxidase. This enzyme was the first documented biologic source of the superoxide radical (McCord and Fridovich, 1968). The enzyme is synthesized as xanthine dehydrogenase. In ischemic tissue, a conversion of xanthine dehydrogenase to xanthine oxidase occurs, probably by a calcium activated protease (McCord, 1985). Both hypoxanthine and xanthine serve as oxidizable purine substrates for xanthine oxidase. Our experiments in the isolated rat heart and the closed chest pig model show that these substrates are available in increasing amounts during ischemia and reperfusion (Appendix, III and VI). Hence, during ischemia two important changes occur in the myocardial tissue: a new enzyme activity appears, along with its required substrates. The remaining substrate required for oxidase activity, molecular oxygen, is supplied upon reperfusion. This sequence of events may result in a burst of free radical formation at a moment when the major defence mechanisms against these radicals are impaired. This imbalance between the formation and scavenging of free radicals has been shown to disrupt calcium transport by the cardiac sarcoplasmic

reticulum (Hess et al., 1981). As described, the unfavourable redistribution of calcium may be an important factor leading to the occurrence of arrhythmias upon reperfusion (see also section A).

At present, reports are beginning to appear which link free radicals to the occurrence of reperfusion arrhythmias (Manning et al., 1984). In this study inhibition of xanthine oxidase with allopurinol resulted in a reduction of arrhythmias, especially upon reperfusion. However, allopurinol could also protect by sparing purines for ATP resynthesis (DeWall et al., 1971).

E. ATP and reperfusion arrhythmias

According to the definition of ischemia as "an imbalance between coronary blood flow and myocardial demand" (Hearse, 1984) tissue oxygen levels will decrease rapidly in the ischemic zone. Consequently, mitochondrial function is impaired (Wilson et al., 1977) and ATP production shifts from aerobic to anaerobic pathways resulting in accelerated glycolysis (Apstein et al., 1977; Neely et al., 1975; Kloner et al., 1975; Opie, 1976; Rovetto et al., 1975). Despite this enhanced glycolytic activity ATP production cannot meet the tissue demand (Hearse; 1979) and cellular ATP-content of the myocardial cells declines (Neely et al., 1973; Hearse et al., 1977; Jennings and Reimer, 1981; Janse et al., 1979; Kloner and Braunwald, 1980; Schaper et al., 1979). This is preceded by a rapid decrease in creatine-phosphate due to its rapid conversion into ATP (Hearse et al., 1977; Hearse, 1979; Hearse et

al., 1983). Concomitant with the decrease in ATP, cellular ADP levels increase transiently. ADP is then converted to AMP and ATP, by adenylate kinase. AMP is then either deaminated to IMP or dephosphorylated to adenosine that is further catabolized to inosine, hypoxanthine and xanthine (Harmsen, 1984; Foker et al., 1980; Olsson, 1970). Since these purines can pass the cell membrane, they will appear in the coronary venous effluent (De Jong et al., 1983; de Jong et al., 1977; Kugler, 1979) where adenosine causes coronary vasodilation (Berne, 1980). Because of the fast exchange between intra- and extracellular nucleosides and oxypurines (Harmsen, 1984; Plageman and Wohlheuter, 1983) and because a slight decrease in ATP results in an immediate rise in AMP catabolites (Wilson et al., 1977; Thompson et al., 1980) it is plausible that AMP catabolites in the coronary effluent may reflect myocardial ATP breakdown (Harmsen, 1984) and thus can be used as a biochemical marker for the extent of ischemia (De Jong et al., 1983; Harmsen, 1984). The metabolic breakdown of ATP into its metabolites and the leakage of these catabolites into the extracellular space will eventually lead to a depletion of the total adenine nucleotide pool. It has been shown that this depletion is correlated with the extent of irreversible ischemic injury (Jennings and Reimer, 1981).

Successful restoration of the arterial flow to reversibly injured ischemic tissue results in reoxygenation of the cells and removal of waste products. Cellular viability will be preserved and the ATP content partially restored. Contractile function however, remains depressed (Apstein et al., 1978). The mitochondria are activated and creatine phosphate levels are restored to even supernormal levels (Ichichara and

Abiko, 1984; Flaherty et al., 1982). The adenylate charge returns to normal whithin three minutes. Virtually all of the increase in ATP comes from phosphorylation of ADP and AMP in the ischemic tissue (Jennings et al., 1984). Further resynthesis of adenine nucleotides occurs very slowly, since AMP-catabolites are washed out during ischemia and during the early phase of reperfusion (De Jong et al., 1983; Reimer et al., 1981). Thus, although the cell is able to function again, ATP levels remain critically low during a prolonged period (Braunwald and Kloner, 1982; Kloner et al., 1981).

Reperfusion of the myocardium after irreversible ischemic injury, i.e. with low ATP, high lactate and malfunctioning mitochondria, results in a massive calcium influx (See section A). The myocytes swell explosively and exhibit disruption of the sarcolemma, massive contraction bands develop and the mitochondria are loaded with calcium phosphate (Ganote et al., 1975; Jennings et al., 1975; Hearse et al., 1975; Schaper et al., 1979).

There is a striking inverse correlation between the cytosol calcium during reperfusion and the ATP levels at the end of the ischemic period (Nayler, 1982). As already pointed out in section A of this chapter these increased levels of cytosolic calcium may be responsible for arrhythmias in the early phase of reperfusion. Whether ATP levels are directly involved in this process is not known at present, mainly because ATP is compartimentalised in the cell (Hearse, 1979). Furthermore, there is indirect evidence showing that recovery of the ventricular multiple response threshold is also related to mitochondrial

function, i.e. ATP production, after reperfusion (Sugiyama et al., 1980a).

Finally, ischemic ATP breakdown is a time-dependent process (Hearse et al., 1983) and the rate of this process will be different for the different ischemic zones (Dennis et al., 1983). Therefore, depending on the severity of ischemia, both reversible and irreversible damaged cells wil be present upon reperfusion after 15 to 60 min of ischemia. Reperfusion will suddenly increase this heterogeneity and thus form the basis for reentry mechanisms (see chapter I).

CHAPTER IV

PHARMACOLOGICAL INTERVENTION

The previous chapter showed us that a cascade of events is involved in the process of ischemia and subsequent reperfusion, making it an exceedingly complex process. On the other hand this multifactorial aspect offers the opportunity for a broad range of drugs to affect the process. Nevertheless, the comparative efficacy of various drugs on reperfusion arrhythmias is not well documented. Furthermore, it is often difficult to discriminate between direct anti-arrhythmic effects and indirect protective effects of the drugs. In the following sections we will discuss three different classes of drugs which were investigated in our reperfusion models.

A. Calcium antagonists

Slow-response action potentials, arising in ischemic ventricular cells, are currently considered to form an important electrophysiological mechanism underlying the development of ventricular fibrillation during acute myocardial ischemia or infarction (Sperelakis, 1984; Opie et al., 1979). However, calcium antagonists do not appear to be universally effective as anti-arrhythmic agents in the clinical

setting (Dargie et al., 1981; Opie, 1980; Henry, 1980). Due to their poor efficacy in established ventricular arrhythmias, the clinical utility of calcium antagonists as anti-arrhythmic agents appears at present to be limited to the treatment of specific forms of atrial arrhythmias (Dargie et al., 1981; Opie, 1984).

As far as reperfusion-induced arrhythmias are concerned, no systematic clinical studies are known at present. Reports on the experimental effects of calcium antagonists on reperfusion arrhythmias are conflicting. The most widely studied calcium antagonist is verapamil. This compound has been shown to be effective preventing ventricular fibrillation during reperfusion in dogs (Brooks et al., 1980; Ribeiro et al., 1981), in the rat (Winslow et al., 1983) and in the pig (Bergey et al., 1984). In contrast Naito and coworkers (1981) failed to demonstrate any beneficial effects of verapamil. Our own limited experience with verapamil in the isolated rat heart suggests that verapamil has some effect on reperfusion arrhythmias, which becomes more pronounced in the presence of adrenaline (van Gilst et al., 1983a). Nifedipine, one of the most potent calcium antagonists (Nayler, 1980a), has also varying results. In the anaesthetized dog, both failure (Ribeiro et al., 1981; Sheehan and Epstein, 1982) and success (Coker and Parratt, 1983a) were reported in relation to the effect of nifedipine on reperfusion-arrhythmias. The latter authors showed that the late administration of nifedipine failed to reduce the incidence of ventricular fibrillation following reperfusion (Coker and Parratt, 1985). Nifedipine administration to the pig, immediately prior to reperfusion was effective in reducing reperfusion arrhythmias (Verdouw

et al., 1981). In the isolated rat heart nifedipine did not appear to reduce the incidence of reperfusion arrhythmias (Winslow et al., 1983; Manning et al., 1983) This same lack of effect was found in the in vivo rat model (Kane et al., 1984). The calcium antagonist, diltiazem, was effective in the isolated rat heart (Winslow et al., 1983; van Gilst et al., 1985b) and the anaesthetized pig (van Gilst et al., 1985b) and ineffective in the dog (Sheehan et al., 1982).

An additional complication to the interpretation of these contradictory results is the variation in time of administration of the drugs. Many investigators have shown that calcium antagonists, given before or during ischemia, have an ATP-sparing effect (Henry, 1980; Henry and Wahl, 1983; Nayler et al., 1980; Perez et al., 1980; Weishaar et al., 1979; Weishaar and Bing, 1980). The precise explanation for this effect is not known yet. Pretreatment of the heart with calcium antagonists will reduce cardiac work via their negative inotropic and chronotropic effects (Fleckenstein and Fleckenstein-Grün, 1980; Nayler, 1980a). Therefore, ATP- and oxygen demand will be reduced before and during ischemia and, as a consequence, the deleterious effects of ischemia delayed. Another possibility is an enhanced preservation of mitochondrial function during ischemia, associated with a reduction in mitochondrial calcium overload (Nayler, 1980b). In our experiments in the isolated rat heart a dose-dependent ATP-sparing effect was observed (Appendix VI). This ATP-sparing effect paralleled the reduction in reperfusion arrhythmias suggesting that the antiarrhythmic effects of calcium antagonists, at least of diltiazem, could be largely explained by an anti-ischemic effect.

Many important questions remain, including whether calcium antagonists have any effect when applied upon reperfusion. In the diltiazem study (Appendix VI) the efficacy of this compound in decreasing reperfusion arrhythmias was markedly reduced when applied during reperfusion only, but a significant effect persisted as far as the duration of reperfusion induced ventricular fibrillation was concerned. This reduction suggests that after 15 min ischemia at least part of the pathophysiological processes occur via the slow channels and that calcium overloading is not solely due to a nonspecific calcium entry via leaky disrupted membranes (Shine, 1978).

The observed effect could be explained by the two stage concept of reperfusion events as proposed by Nayler (1985). In this concept the initial event (stage one) is the entry of a small amount of calcium in the presence of low levels of tissue ATP (Hearse, 1977) and a poorly functioning sarcoplasmatic reticulum (Hess et al., 1981) due to a short period of ischemia. This initial rise in cytosolic calcium could, theoretically, increase phospholipase activity and contraction in turn causing ultrastructural damage. This damage might then trigger a secondary and relatively larger gain in calcium (stage two). It is conceivable that the initial calcium entry occurs via the normal calcium entry pathways such as the slow channels (see also chapter III, section B). Slowing down or even prevention of the initial event will alter the development of tissue heterogeneity which may form the basis of reperfusion arrhythmias (Chapter I).

Another contributing effect relative to the ability of calcium antagonists to reduce the incidence of reperfusion arrhythmias may be

their effect on cardiac noradrenaline depletion during postischemic reperfusion (Nayler and Sturrock, 1983; Nayler and Sturrock, 1985). It is known that catecholamines generate slow action potentials, particularly under the conditions of a raised level of extracellular potassium encountered during ischemia (Kleber, 1984; Weiss and Shine, 1982). Thus, calcium antagonists may not only directly prevent calcium entry but may also indirectly prevent the release of a calcium entry promoting agent, noradrenaline. Hence, the electrophysiologic derangements induced by catecholamines (Penny, 1984) are prevented.

Apart from the slow channel blocking effect, some calcium antagonists exhibit additional properties which may modulate reperfusion arrhythmias. For instance, verapamil and diltiazem also interact with alpha-adrenoceptors (Nayler et al., 1982; Karlinger et al., 1982) and these are believed to play a role in the generation of reperfusion-induced arrhythmias (see Chapter III). Furthermore, some calcium antagonists supress the fast inward sodium current (Kass and Tsien, 1975: Labrid et al., 1979). However, to date there is no really conclusive evidence that any fast sodium channel inhibitor is able to reduce the incidence of reperfusion-induced ventricular arrhythmias (Manning and Hearse, 1984) There is however some indication that these arrhythmias are susceptible to fast sodium inward blockade (Kane et al., 1984).

B. Prostacyclin and stable analogues

In Chapter III, section B we discussed already the possible role of prostaglandins in the genesis of reperfusion arrhythmias. The inverse relationship between prostacyclin release from the ischemic myocardium and reperfusion arrhythmias was mentioned (Coker et al., 1981b). The same group demonstrated that a pharmacologically induced increase in prostacyclin concentrations by nafazatrom (Coker and Parratt, 1983c) or the administration of iloprost (Coker and Parratt, 1983b), reduced the incidence of reperfusion-induced ventricular fibrillation in dogs. According to these authors it could be hypothetised that upon reperfusion platelet emboli are flushed downstream leading to severe, but localized, mechanical obstruction of the coronary microcirculation (Parratt and Coker, 1985). Flow reduction in such localized areas of the ventricular wall, adjacent to normally or hyperperfused regions could lead to conditions favouring re-entry circuits (see also Chapter I). Locally increased prostacyclin concentrations could induce vasodilation (Lefer et al., 1978), reduce platelet adherence to the damaged vessel wall and resolve platelet aggregates (Aiken et al., 1979). Although this is certainly a plausible explanation for the observed effects in an in vivo model, we detected a reduced incidence and duration of ventricular fibrillation following the administration of iloprost in isolated rat hearts (van Gilst et al., 1985b; Appendix VII). This indicates that prostacyclin, or at least its stable analogue, also has antiarrhythmic properties in the absence of platelets and at doses that do not cause vasodilation. Furthermore, it was concluded from our studies (Appendix

II and VII) that iloprost exerts its main effect by reducing cellular damage during ischemia, probably by preserving membrane integrity. This direct, flow independent cell protection has also been shown by other investigators (Schrör et al., 1981; Smith et al., 1984; Nayler et al., 1984; de Langen et al., 1985). Via this mode of action, tissue heterogeneity will be altered in a favourable way just prior to reperfusion. This will lead to a reduced incidence of reperfusion arrhythmias (see also Chapter I). Furthermore, apart from protecting myocardial cell membranes prostacyclin and its analogues may also preserve membrane function in cardiac adrenergic nerve endings. In this case a reduction in locally released noradrenaline will occur (Schrör et al., 1982; Schrör and Funke, 1985) and reperfusion arrhythmias will therefore be attenuated (see Chapter III, section C).

C. Converting enzyme inhibitors

The extent to which the renin-angiotensin system participates in the process of myocardial ischemia and reperfusion is unknown. Recent evidence indicates that the renin-angiotensin system is activated by acute coronary occlusion in vivo (Liang et al., 1982; Ertl et al., 1983). Since blockade of this system lowers systemic blood pressure and improves cardiac output (Liang et al., 1982), it may play a role in the extension of ischemic damage of the myocardium (Hock et al., 1985; Lefer and Peck, 1984; Ertl et al., 1982). It has also been shown that converting enzyme inhibitors, at least captopril, interfere with the

response to noradrenaline by an angiotensin-II-independent mechanism (Clough et al., 1982; Saruta et al., 1982) and that these agents may facilitate prostacyclin synthesis (Swartz et al., 1980; Düsing et al., 1983; Mullane and Moncada, 1980). These factors both interfere with the genesis of reperfusion arrhythmias (see Chapter III). For this reason we used isolated rat hearts to investigate whether converting enzyme inhibitors have any direct effects on reperfusion arrhythmias, independent of changes in hemodynamics. It appeared that captopril effectively reduced the incidence of ventricular fibrillation upon reperfusion (van Gilst et al., 1984a, Appendix III and IV) in a dose dependent manner (de Graeff et al., 1986). Furthermore, this property was shared by another angiotensin converting enzyme inhibitor, HOE 498, but not by a third compound of this group, enalapril. The effects of HOE 498 were limited to the active enzyme inhibiting form and were not found for the inactive prodrug (Appendix IV). The effects of captopril were associated with an abolishment of noradrenaline overflow upon reperfusion. Simultaneous administration of indomethacin attenuated the effects of captopril.

Several mechanisms may be responsible for the observed effects. An interference with the production of angiotensin II seems unlikely in our model since we were not able to demonstrate the presence of this compound in the coronary effluent of control hearts with a sensitive radio-immunoassay. As mentioned above, captopril has been shown to possess direct antiadrenergic properties (Saruta et al., 1982; Clough et al., 1982). According to the findings of Sheridan and colleagues (1980), blockade of alpha-adrenergic receptors, which are increased in number

during ischemia (Corr et al., 1981) will lead to a reduced incidence of reperfusion arrhythmias. However, our results show that the reduction in reperfusion arrhythmias is associated with a reduced noradrenaline overflow. This suggest that captopril is active at the presynaptic site a finding, which is difficult to explain in terms of a blockade of adrenergic receptors. Preservation of adrenergic nerve endings during ischemia has been demonstrated in the presence of increased prostacyclin concentrations (Schrör and Funke, 1985; see also Chapter III). This situation may be obtained by captopril treatment, since captopril can induce an increase in prostacyclin synthesis in the in vitro situation (Düsing et al, 1983) probably by a reduction in bradykinin inactivation (Erdos, 1976) by kininase II, an enzyme which is identical to converting enzyme (Erdos, 1975). This is an attractive hypothesis since it also explains the results observed during simultaneous indomethacin treatment. It is also in agreement with the finding that the inactive enzyme inhibiting form of HOE 498 has no effect. The ineffectivity of enalapril is more difficult to explain but may be due to the concentration used, since a slight but not significant reduction in purine overflow and incidence of reperfusion arrhythmias were found.

The efficacy of captopril in preventing reperfusion arrhythmias may also be due to its potential as scavenger of free radicals since captopril possesses a sulfhydryl-group. This chemical structure enables captopril to act in a manner which is comparable with that of the physiologic sulfhydryl containing scavenger, glutathione. The availability of glutathione is reduced during ischemia and the first minutes of reperfusion (see chapter III section E) and administration of

glutathione upon reperfusion has been shown to reduce reperfusion arrhythmias (Woodward and Zakaria, 1985). Nevertheless, HOE 498 also reduces the incidence of reperfusion arrhythmias although it lacks pronounced scavenging properties. Thus, only part of the efficacy of captopril can be ascribed to this mechanism.

CHAPTER V

CLINICAL RELEVANCE

In the previous chapters it was endeavoured to summarize the possible mechanisms underlying the genesis of reperfusion arrhythmias, the cellular events modulating these events and finally the potential pharmacological interventions. The data discussed in these chapters were largely obtained from animal experiments. However, there are only few reports which confirm these experimental data on reperfusion arrhythmias in clinical practice. One of the main problems is that direct identification of occlusion and reperfusion phases is very difficult in man. Chapter one discribes some human models in which sudden decrease of coronary flow persists for several minutes, following which flow is reestablished. Probably the most frequent spontaneously occurring sequence of events is a spasm of a coronary artery and its subsequent relief. Arrhythmias associated with this situation are suggested as a cause of sudden cardiac death in man (Elharrar and Zipes, 1977). A spasm of a large subepicardial coronary artery is currently considered the main pathophysiologic mechanism of variant angina (Oliva et al., 1973; Maseri et al., 1977a). Since this anomaly is relatively easy to demonstrate by angiography, most clinical data on reperfusion arrhythmias are obtained in these patients with variant angina (Tzivoni

et al., 1983; Previtali et al., 1983; Gabliani et al., 1985; Kerin et al., 1983).

During recent years it has been suggested that coronary artery spasm is far more common in angina than originally thought and also encountered in patients with stable angina (Conti, 1985; Maseri et al., 1977b; Maseri et al., 1985). Patients suffering from this form of angina have a recognizable threshold of exertion that they can never exceed without developing angina. In addition, they have episodes of angina at rest or at levels of exertion that are usually quite well tolerated. The term mixed angina was proposed for this form (Maseri, 1980) and two elements are thought to determine this syndrome. The first is a critical coronary obstruction that sets the limit of maximal residual coronary flow reserve, i.e. the level of effort or of oxygen demand that the patient cannot exceed without developing ischemia. This maximal residual coronary flow reserve results from the balance between the severity of coronary obstruction and collateral blood supply. The second is a number of different mechanisms that can suddenly and transiently reduce coronary flow to a variable extent. When flow is reduced below the resting needs, spontaneous ischemia at rest occurs.

The mechanisms that may be responsible for the transient impairment of coronary blood flow caused by increased vascular tone include supersensitivity of a segment of a large epicardial coronary artery. This causes a total or subtotal occlusion in response to stimuli that will normally change vascular diameter only to a modest degree. This supersensitivity may be mediated by alterations in the membrane receptors of smooth muscle cells. Radioligand binding experiments and

receptor autoradiographic studies with atherosclerotic rabbit aortas have revealed an increased abundance of serotonergic and alpha adrenergic receptors localized in the inner media and intima (Nanda and Henry, 1982). It has also been suggested that patients with atherosclerosis react excessively to the cold pressor test, a phenomenon that can indicate an altered serotonergic or noradrenergic responsiveness (Voudoukis, 1970). Furthermore, increased vasomotor tone at the site of a subendocardial plaque, which reduces the lumen to a critical or subcritical extent, may play an important role (MacAlpin, 1980). The presence of atheroma makes the occurrence of endothelial damage very likely. Furchgott and his colleagues demonstrated that an important factor governing the reactivity of vascular smooth muscle to vasoactive agents is the integrity of the endothelium (Furchgott, 1983). They proposed that interaction with specific receptors on the endothelial cells led to the release of a factor, termed endothelial relaxing factor (EDRF), which then acts on the arterial smooth mucle cells causing them to relax. Possibly, under normal circumstances vasoconstrictor agents activate endothelial cells and this activation leads to the release of EDRF which inhibits smooth muscle cell contraction and hence act as a "brake" to modulate the effects of such agonists (Egleme et al., 1984). If integrity of endothelial cells is a prerequisite for a normal functioning of EDRF, this could explain that, in the presence of naturally occuring spasmogens, such spasm is restricted to certain atherosclerotic regions of coronary arteries in patients with mixed angina.

Apart from loss of EDRF due to endothelial damage there will also be a loss of prostacyclin which is synthesized by these cells. Loss of prostacyclin could lead to localised platelet adherence, to the release of platelet derived vasoconstrictors such as thromboxane A2 and serotonin, and to platelet aggregation and transient intravascular plugging by deposited platelets. It has been shown that angina, including vasotic angina, can be associated with the release of thromboxane A2 into the coronary venous blood (Robertson et al., 1981; Hirsh et al., 1981). However, although platelets may play a role in precipitating or aggravating coronary artery spasm, intravenous prostacyclin or cyclooxygenase inhibition have thus far not been shown to exert beneficial effects (Chierchia et al., 1982a; Chierchia et al., 1982b; Robertson et al., 1981). Furthermore, the first results with an antagonistic serotonin vascular receptor blocker in a small number of patients with variant angina were disappointing, making it unlikely that serotonin plays an important role in vasospastic angina (De Caterina et al., 1984).

Although occlusion and reperfusion phases cannot be directly indentified in man, experimental and clinical studies have shown that coronary artery occlusion is associated with ST-segment elevation (Corbalan et al., 1976; Maseri et al., 1979), and reperfusion with a rapid return of the ST-segment to baseline values (Corbalan et al., 1976; Chierchia et al., 1980). Therefore in some studies monitoring of ST segment changes was used to identify periods of occlusion and subsequent reperfusion in patients with vasospastic angina. The incidence of reperfusion arrhythmias (i.e. those occurring during the

resolution of, or soon after, ST-segment normalisation) was observed in 20-37% of the patients with ventricular arrhythmias (Previtali et al., 1983; Kerin et al., 1983). Reperfusion arrhythmias were characterised by ventricular bigeminy and ventricular tachycardia or ventricular fibrillation without prodromal ectopic activity. Furthermore, they appeared to be correlated with the severity of ischemia, as measured by the degree of ST-segment elevation, and with the duration of ischemia (Previtali et al., 1983). The relatively low incidence of reperfusion arrhythmias in these patients was probably due to the short duration of ischemia (5-15 minutes). The time dependency of reperfusion arrhythmias was already discussed in earlier chapters and in the experimental setting longer periods of 15-20 min of ischemia certainly result in more and severe arrhythmias upon reperfusion.

Although the role of reperfusion in patients is still under investigation, the few reports on reperfusion phenomena which appeared until now already have therapeutic implications. They indicate that patients with transient episodes of ischemia associated with severe ventricular arrhythmias have a high mortality and are likely to die suddenly (Miller et al., 1982). These patients form a high-risk group in whom prevention of life-threatening arrhythmias is of utmost importance. In chapter IV it was already discussed that in experimental studies various antiarrhythmic drugs used in the prevention of reperfusion arrhythmias have led to conflicting results (Naito et al., 1981; Stewart et al., 1980). In patients a dearrangement of regulatory function of vasomotor activity leading to coronary artery spasm is probably the most common underlying mechanism of transient ischemia resulting in

ventricular arrhythmias. Therefore, a rational approach should aim at prevention of spasm rather than at treatment of arrhythmias once ischemia has developed. The three classes of drugs discussed in chapter IV all affect in one way or another coronary vasomotor function. Converting enzyme inhibitors inhibit the production of angiotensin II and thus reduce local sympathetic activity. Furthermore, these agents may facilitate prostacyclin synthesis (Düsing et al., 1983) which, as well as analogues like iloprost, induces vasodilation and reduces platelet aggregation. The efficacy of calcium antagonists for the treatment of vasospastic angina has been widely confirmed (Kimura and Kishida, 1981; Antma et al., 1980). In our experimental studies all these agents reduced the incidence of reperfusion arrhythmias after total coronary occlusion (see Chapter IV). Thus, this effect was independent of their potency to reduce dynamic coronary obstruction. Therefore, in the clinical situation these agents may influence reperfusion arrhythmias via at least two different pathways; indirectly by preventing the sequence of ischemia and reperfusion and directly as shown in animal models described in this thesis. The clinical relevance of our work was demonstrated by our study, which showed that diltiazem (240 mg/day) was very effective in preventing reperfusion induced ventricular tachycardias in a patient with exercise related reperfusion phenomena (Appendix VIII).

In conclusion, the situation regarding reperfusion arrhythmias in the clinical situation is open for further investigation. Especially the lack of simple methods to identify reperfusion phenomena in patients and the unpredictable occurrence of transient ischemic periods offer

difficulties in assessing the precise role of reperfusion arrhythmias in ischemic heart disease. The efficacy of agents in preventing reperfusion arrhythmias in man can only be properly assessed when these problems are resolved.

CHAPTER VI

SUMMARY AND CONCLUDING REMARKS

Fifty years ago already it was observed that a sudden restoration of antegrade flow in the ischemic myocardium can lead to life-threatening arrhythmias. The implications of this observation were only fully understood during the last decade when, thanks to the improvement of angiographic methods, reperfusion could more easily be demonstrated in man. At present it is generally accepted that reperfusion phenomena play an important role in ischemic heart disease in man and probably the arrhythmias, which are a consequence of reperfusion, are responsible for a large percentage of sudden cardiac deaths. It was the aim of the experiments described in this thesis to further elucidate the mechanisms of reperfusion arrhythmias and their pharmacological modulation.

In Chapter I concepts and recent findings concerning the electrophysiology of reperfusion arrhythmias are outlined. Like arrhythmias induced by ischemia, reperfusion arrhythmias can be divided into distinct categories arising at different times and occurring as a result of distinct mechanisms. One type, occurring during the first seconds of reperfusion, has a high incidence of ventricular fibrillation and is proposed to be due to reentry. The second type, occurring several minutes after the onset of reperfusion is associated with a significantly lower incidence of ventricular fibrillation and is thought to be due to enhanced automaticity. Both ischemia induced arrhythmias

and reperfusion arrhythmias are the consequence of metabolic changes in the cardiac cells in the area at risk. Probably the most important difference between these phenomena is the velocity of these changes.

The research of reperfusion arrhythmias in man is often hampered by the limitations of assesing the moment of reperfusion. In order to circumvent this problem animal models can be used in which the duration and severity of ischemia can be strictly controlled.

Chapter II describes the models used in our studies. These ranged from an isolated rat heart to an in vivo monkey preparation, each of which has its inherent advantages and disadvantages, as discussed in this chapter.

As mentioned above reperfusion arrhythmias are a consequence of metabolic changes of the cardiac cells in the ischemic and subsequently reperfused area. These changes and their role in arrhythmogenesis upon reperfusion are discussed in Chapter III. Data are presented which emphasize the importance of the process of calcium overloading as a trigger for reperfusion arrhythmias. The involvement of catecholamines, prostaglandins and free radicals in the arrhythmogenesis upon reperfusion are also demonstrated. However, the underlying mechanisms and their place in the cascade of events taking place upon reperfusion is not fully understood at present.

Subsequently, ATP catabolism is discussed in relation to adenosine and catabolite release in the coronary-venous system. Determination of the purines in the extracellular fluid gives a continuous insight in intracellular ATP metabolism during ischemia and reperfusion. The overflow-kinetics can be used to estimate the seperate contribution of both phenomena to ATP-catabolism. The level of myocardial ATP is an

important determinant for cell function and a relationship between ATP loss and reperfusion arrhythmias is demonstrated.

In Chapter IV the pharmacological intervention of reperfusion arrhythmias is discussed. Three classes of drugs, calcium antagonists, prostacyclin analogues and angiotensin converting enzyme inhibitors are investigated in our different reperfusion models. The reduction in reperfusion arrhythmias by the calcium antagonist diltiazem could be largely explained by its ATP-sparing effect during ischemia. However, when applied during reperfusion only, there was still a significant effect on the duration of reperfusion induced ventricular fibrillation. This suggests that diltiazem is able to influence the development of reperfusion damage. The stable prostacylin analogue iloprost exerted its main effect by reducing cellular damage during ischemia, probably by preserving membrane integrity.

It appeared that the angiotensin converting enzyme inhibitors, captopril and HOE 498, effectively reduced the incidence of ventricular fibrillation upon reperfusion. The effects of captopril were associated with an abolishment of noradrenaline overflow upon reperfusion. Simultaneous administration of indomethacin attenuated the effects of captopril. It is postulated that the beneficial effects of captopril are the result of an interference with the metabolism of prostaglandins.

In Chapter V the available data on reperfusion arrhythmias in the clinical situation are summarized. The lack of simple methods to identify reperfusion phenomena in patients and the unpredictable occurrence of transient ischemic periods cause difficulties in assessing the precise role of reperfusion arrhythmias in ischemic heart disease at present. These problems must be resolved before the efficacy of agents

in preventing reperfusion arrhythmias in man can be assessed properly. Insight in these phenomena seems mandatory to combat sudden cardiac death, which remains the challenge of contemporary cardiology.

REFERENCES

Abrahamsson T, Almgren O, Carlsson L (1983) Ischemia-induced noradrenaline release in the isolated rat heart: influence of perfusion substrate and duration of ischemia. J Mol Cell Cardiol 15: 821-830

Abrahamsson T, Almgren O, Carlsson L (1984) Wash-out of noradrenaline and its metabolites by calcium-free reperfusion after ischaemia: support for the concept of ischaemia-induced noradrenaline release. Br J Pharmacol 81: 22-24

Aiken JW, Gorman RR, Shebushi RJ (1979) Prevention of blockage of partially obstructed coronary arteries with prostacyclin correlates with inhition of platelet aggregation. Prostaglandins 17: 483-494

Alexander RW, Kent KM, Piscino JJ, Keiser HR, Cooper T (1975) Regulation of post occlusive hyperemia by endogenously synthetised prostaglandins in the dog heart. J Clin Invest 55: 1174-1181

Antman E, Muller JE, Goldberg S, MacAlpin R, Rubenfine M, Tabatznid B, Liang C, Heupler F, Achuff S, Reichek N, Geltman E, Kerin NZ, Neff RK, Braunwald E (1980) Nifedipine therapy for coronary artery spasm: experience in 127 patients. N Engl J Med 302: 1269-1273

Apstein CS, Deckelbaum L, Mueller M, Hagopian L, Hood WB (1977) Graded global ischemia and reperfusion. Circulation 55: 864-872

Apstein CS, Deckelbaum L, Hagopian L, Hood WB (1978) Acute cardiac ischemia and reperfusion: contractility, relaxation and glycolysis. Am J Physiol 235: H637-H648

Araki H, Lefer AM (1980) Role of prostacyclin in the preservation of ischemic tissue in the perfused cat heart. Cir Res 47: 757-763

Axelrod PJ, Verrier RL, Lown B (1975) Vulnerability to ventricular fibrillation during acute coronary arterial occlusion and release. Am J Cardiol 36: 776-782

Bashe WJ, Baba N, Keller MD, Geer JC, Anthony, JR (1975) Pathology of atherosclerotic heart disease in sudden death. II. The significance of myocardial infarction. Circulation 52, III-63

Baba N, Bashe WJ, Keller MP, Geer JK, Anthony JR (1975) Pathology of atherosclerotic heart disease in sudden death I Organizing thrombosis and acute coronary vessel lesions. Circulation 52, III-53

Balke CW, Kaplinsky E, Michelson EL, Naito M, Dreifus LS (1981) Reperfusion ventricular tachyarrhythmias: correlation with antecedent coronary artery occlusion tachyarrhythmias and duration of myocardial ischemia. Am Heart J 101: 449-456

Becker RHA, Schölkens BA, Metzger M, Schulze KJ (1984) Pharmacological properties of the new orally active converting enzyme inhibitor HOE 498. Drug Research, 34 (II) 1411-1416

Bergey JL, Nocella K, McCallum JD (1982) Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. Eur J Pharmacol 81: 205-216

Bergey JL, Wendt RL, Nocella K, McCallum JD (1984) Acute coronary artery occlusion-reperfusion arrhythmias in pigs: antiarrhythmic and antifibrillatory evaluation of verapamil, nifedipine, prenylamine and propranolol. Eur J Pharmacol 97: 95-103

Berger HJ, Zaret BL, Speroff L, Cohen LS, Wolfson S (1977) Cardiac prostaglandin release during myocardial ischemia induced by atrial pacing in patients with coronary artery disease. Am J Cardiol 39: 481-486

Berne RM (1980) The role of adenosine in the regulation of coronary blood flow. Circ Res 47: 807-813

Bonvallet R, Rongier O, Tourneur Y (1984) Role of Na - Ca exchange in the calcium paradox in frog auricular trabeculae. J Mol Cell Cardiol 16: 623-632

Borda L, Schuchleib R, Henry PD (1977) Effects of potassium on isolated canine coronary arteries: modulation of adrenergic responsiveness and release of norepinephrine. Circ Res 14: 778-786

Bourdillon PD, Poole-Wilson PA (1981) Effects of ischemia and reperfusion on calcium exchange and mechanical function in isolated rabbit myocardium. Cardiovasc Res 15: 121-130

Bralet J, Didier JP, Moreau D, Opie LH, Rochette L (1985) Effect of alpha-adrenoceptor antagonists (phentolamine, nicergoline and prazosin) on reperfusion arrhythmias and noradrenaline release in perfused rat heart. Br J Pharmac 84: 9-18

Braunwald, E, Kloner, RA (1982) The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 66: 1146-1149

Brooks H, Al-Sadir J, Schwartz J, Rich B, Harper P, Resnekov L (1975) Biventricular dynamics during quantitated anteroseptal infarction in the porcine heart. Am J Cardiol 36: 365-374

Brooks WW, Verrier RL, Lown B (1980) Protective effect of verapamil on vulnerability to ventricular fibrillation during myocardial ischaemia and reperfusion. Cardiovasc Res 14: 295-302

Chierchia S, Brunelli C, Simonetti I, Lazzari M, Maseri A (1980) Sequence of events in angina at rest: primary reduction in coronary flow. Circulation 61: 759-768

Chierchia S, Patrono C, Crea F, Ciabattoni G, De Catarina R, Cinotti GA, Distante A, Maseri A (1982a) Effects of intravenous prostacylcin in variant angina. Circulation 65: 470-477

Chierchia S, De Catarina R, Crea F, Patrono C, Maseri A (1982b) Failure of thromboxane A2 blockade to prevent attacks of vasospastic angina. Circulation 66: 702-705

Clough DP, Collis MG, Conway J, Hatton R, Keddie JR (1982) Interaction of angiotensin-converting enzyme inhibitors with the function of the sympathetic nervous system. Am J Cardiol 49: 1410-1414

Clusin WT, Buchbinder M, Harrison DC (1983) Calcium overload, 'injury' current, and early ischaemic cardiac arrhythmias - a direct connection. Lancet 17: 272-273

Coker SJ, Parratt JR, Ledingham IMcA, Zeitlin IJ (1981a) Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. Nature 291: 323-324

Coker SJ, Ledingham IMcA, Parratt JR, Zeitlin IJ (1981b) Aspirin inhibits the early myocardial release of thromboxane B2 and ventricular ectopic activity following acute coronary artery occlusion in dogs. Br J Pharmacol 72: 593-595

Coker SJ, Parratt JR, Ledingham IMcA, Zeitlin IJ (1982a) Evidence that thromboxane contributes to ventricular fibrillation induced by reperfusion of the ischaemic myocardium. J Mol Cell Cardiol 14: 483-485

Coker SJ, Fagbemi O, Parratt JR (1982b) Lidoflazine in the early stages of acute myocardial ischaemia. Br J Pharmacol 72: 347-354

Coker SJ, Parratt JR (1983a) Nifedipine reduces arrhythmias but does not alter prostanoid release during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J Cardiovasc Pharm 5: 406-417

Coker SJ, Parratt JR (1983b) Prostacyclin-antiarrhythmic or arrhythmogenic? Comparison of the effects of intravenous and intracoronary prostacyclin and ZK 36 374 during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J Cardiovasc Pharmacol 5: 557-567

Coker SJ, Parratt JR (1983c) The effects of nafazatrom on arrhythmias and prostanoid release during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J Mol Cell Cardiol 16: 43-52

Coker SJ (1984) Further evidence that thromboxane exacerbates arrhythmias : Effects of UK 38485 during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J Mol Cell Cardiol 16: 633-641

Coker SJ, Parratt JR (1984) The effects of timolol on arrhythmias and prostanoid release during canine myocardial ischaemia and reperfusion. Br J Pharmac 81: 675-684

Coker SJ, Parratt JR (1985) Relationships between the severity of myocardial ischaemia, reperfusion induced ventricular fibrillation, and the late administration of dazmegrel or nifedipine. J Cardiovasc Pharmacol 7: 327-334

Conti CR (1985) Large vessel coronary vasospasm: Diagnosis, natural history and treatment. Am J Cardiol 55: 41B-49B

Corbalan R, Verrier RL, Lown B (1976) Differing mechanisms for ventricular vulnerability during coronary artery occlusion and release. Am Heart J 92: 223

Corr PB, Gillis RA (1978) Autonomic neural influences on the dysrhythmias resulting from myocardial infarction. Circ Res 43: 1-9

Corr PB, Hayman JA, Kramer JB, Kipnis RJ (1981) Increased alpha-adrenergic receptors in ischaemic cat myocardium - a potential mediator of electrophysiological derangements. J Clin Invest 67: 1232-1236

Corr PB, Crafford WA (1981) Enhanced alpha-adrenergic responsiveness in ischemic myocardium: Role of alpha-adrenergic blockade. Am Heart J 102: 605-612

Corr PB, Snyder DW, Lee BI, Gross RW, Keim CR, Sobel BE (1982) Pathophysiological concentrations of lysophosphatides and the slow response. Am J Physiol 243: 187-195

Corr PB, Witkowski FX (1983) Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischaemic myocardium. Circulation 68, suppl 1: 16-24

Corr PB, Witkowski FX (1984) Arrhythmias associated with reperfusion: Basic insights and clinical relevance. J Cardiovasc Pharmacol 6: S903-S909

Crome R, Hearse DJ, Manning A (1983) Relationship between cellular cyclic AMP content and the incidence of ventricular fibrillation upon reperfusion after varying periods of ischaemia. J Mol Cell Cardiol 15 (suppl 1): 180

Culling W, Penny WJ, Lewis MJ, Middleton K, Sheridan DJ (1984) Effects of myocardial catecholamine depletion on cellular electrophysiology and arrhythmias during ischaemia and reperfusion. Cardiovasc Res 18: 675-682

Dargie H, Rowland E, Krikler D (1981) Role of calcium antagonists in cardiovascular therapy. Br Heart J 46: 8

Davey MJ (1980) Relevant features of the pharmacology of prazosin. J Cardiol Pharm 2: S287-301

Davies MJ, Thomas A (1984) Thrombosis and acute coronary artery lesions in sudden cardiac ischemic death. N Eng J Med 310: 1138

De Caterina R, Carpeggiani C, L'Abbate A (1984) A double-blind, placebo-controlled study of ketanserin in patients with Prinzmetal's angina. Evidence against a role for serotonin in the genesis of coronary vasospasm. Circulation 69: 889-894

De Mello, WC (1982) Intercellular communication in cardiac muscle. Circ Res 51: 1-9

Dennis SC, Shattock MJ, Hearse DJ, Ball MR, Sochor M, McLean P (1983) Two different metabolic responses to ischaemia: inherent variability or artefact? Cardiovasc Res 17: 489-498

DeWall RA, Vasko KA, Stanley EL, Kezdi P (1971) Responses of the ischemic myocardium to allopurinol. Am Heart J 82: 362-270

Didier JP, Rochette L, Moreau D, Bralet J (1980) Liberation de noradrenaline et troubles grave du rhythme du coeur isole perfuse travaillant en presence d'acides gras. Influence de la ligature coronaire et de la reperfusion. J Physiol (Paris) 76: 723

Downar E, Janse MJ, Durrer D (1977) The effect of acute coronary occlusion on subepicardial transmembrane potentials in the intact porcine heart. Circulation 56: 217-224

Dunnett JS, Nayler WG (1979) Effect of pH on calcium accumulation and release from isolated fragments of cardiac and skeletal muscle sarcoplasmic reticulum. Biochem Biophys Acta 198: 434-438

Düsing R, Scherag R, Landsberg G, Glanzer K, Kramer HJ (1983) The convertingenzyme inhibitor captopril stimulates prostacyclin synthesis by isolated rat aorta. Eur J Pharmacol 91: 501-504

Ebert PA, Vanderbeek RB, Allgood RJ, Sabiston DC (1970). Effect of chronic cardiac denervation on arrhythmias after coronary artery ligation. Cardiovasc Res 4: 141-147

Eckstein RW (1954) Coronary interarterial anastomoses in young pigs and mongrel dogs. Circ Res 11: 460-465

Egleme C, Godfraind T, Miller RC (1984) Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. Br J Pharmac 81: 16-18

Elharrar V, Zipes DP (1977) Cardiac electrophysiologic alterations during myocardial ischemia. Am J Physiol 232: H329-H345

Ellis EF, Oelz O, Roberts LJ, Payne NA, Sweetman BJ, Nies AS, Oates JA (1976) Coronary arterial smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A2. Science 193: 1135-1137

Erdos EG (1975) Angiotensin I converting enzyme. Circ Res 36: 247-255

Erdos EG (1976) Conversion of angiotensin I to angiotensin II. Am J Med 60: 749-759

Ertl G, Kloner RA, Alexander RW, Braunwald E (1982) Limitation of experimental infarct size by an angiotensin-converting enzyme inhibitor. Circulation 65: 40

Ertl G, Alexander RW, Kloner RA (1983) Interactions between coronary occlusion and the renin-angiotensin system in the dog. Bas Res Cardiol 78: 518

Evans RG, Val-Mejias JE, Kulevich J, Fisher VW, Mueller HS (1985) Evaluation of a rat model for assessing interventions to salvage ischaemic myocardium: effects of ibuprofen and verapamil. Cardiovasc Res 19: 132-138

Farber JL (1981) The role of calcium in cell death. Life Sci 29: 1289-1295

Ferrier GR (1977) Digitalis arrhythmias: Role of oscillatory afterpotentials. Prog Cardiovasc Dis 19: 459

Ferrier GR, Moffat MP, Lukas A (1985) Possible mechanisms of ventricular arrhythmias elicited by ischemia followed by reperfusion. Studies on isolated canine ventricular tissues. Circ Res 56: 184-194

Fleckenstein A, Fleckenstein-Grün G (1980) Cardiovascular protection by calcium-antagonist. Eur Heart J 1 (suppl B): 15-21

Flaherty JT, Weisfeld ML, Buckley BH, Gardner TJ, Gott VT, Jacobus WE (1982) Mechanism of ischemic myocardial cell damage assessed by phosphorus-31 Nuclear Magnetic Resonance. Circulation 65: 561-576

Foker JE, Enzig S, Wang T (1980) Adenosine metabolsim and myocardial preservation. J Thorac Cardiovasc Surg 80: 506-516

Friedrich T, Lichey J, Nigam S, Priesnitz M, Wegscheider K (1985) Follow-up of prostaglandin plasma levels after acute myocardial infarction. Am Heart J 109: 218-222

Fujimoto T, Peter T, Hamamoto H, Mandel WJ (1983) Electrophysiologic observations on ventricular tachyarrhythmias following reperfusion. Am Heart J 105: 201

Furchgott RF (1983) Role of endothelium in responses of vascular smooth muscle. Circ Res 53: 557-573

Gabliani GI, Winniford MD, Fulton KL, Johnson SM, Mauritson DR, Hillis LD (1985) Ventricular ectopic activity with spontaneous variant angina: Frequency and relation to transient ST segment deviation. Am Heart J 110: 40-43

Ganote CE, Liu SY, Safavi S, Kaltenbach JP (1981) Anoxia, calcium and contracture as mediators of myocardial enzyme release. J Mol Cell Cardiol 13: 93-106

Ganote CE, Seabra-Gomes R, Nayler WG, Jennings RB (1975) Irreversible myocardial injury in anoxic perfused rat hearts. Am J Pathol 80: 419-450

Ganten D, Schelling P, Vescei P, Ganten U (1976) Iso-renin of extrarenalorigin The tissue angiotensinogenase systems. Am J Med 60: 760-772

Gauduel Y, Duvelleroy M (1984) Role of oxygen radicals in cardiac injury due to reoxygenation. J Mol Cell Cardiol 16: 459-470

Geary GG, Smith GT, Suehiro GT, McNamara JJ (1982) Failure of nifedipine therapy to reduce myocardial infarct size in the baboon. Am J Cardiol 49: 331

Gilst WH van, Langen CDJ de (1982) Ischemia-reperfusion induced arrhythmias in the isolated rat heart. Pharm Weekblad Sci ed 45: 160

Gilst WH van, Wierenga BJ, Langen CDJ de (1983a) Ischemia and reperfusion induced ventricular arrhythmias; interaction of verapamil and catecholamine. Proc 24th Dutch Fed Meeting 117

Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRM, Langen CDJ de (1983b) Improved functional recovery of the isolated rat heart after 24 hours of hypothermic arrest with a stable prostacyclin analogue (ZK 36 374). J Mol Cell Cardiol 15: 789-792

Gilst WH van, Graeff PA de, Kingma JH, Wesseling H, Langen CDJ de (1984a) Captopril reduces purine loss and reperfusion arrhythmias in the rat heart after coronary artery occlusion. Eur J Pharmacol 100: 113-117

Gilst WH van, Houwertjes MC, Wesseling H (1984b) Reperfusion arrhythmias in the monkey heart after coronary occlusion. Proc 9th IUPHAR congress, MacMillan, London 470

Gilst WH van, Daemen BJC, Langen CDJ de (1984c) Ischemia and reperfusion induced ventricular arrhythmias; role of calcium and magnesium. Proc 25th Dutch Fed Meeting 316

Gilst WH van, Graeff PA de, Wesseling H, Langen CDJ de (1985a) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors. A comparison of captopril, enalapril and HOE 498. J Cardiovasc Pharm, in press

Gilst WH van, Terpstra JA, Langen CDJ de (1985b) Ventricular arrhythmias and purine loss upon reperfusion of ischemic myocardium: Comparison of ZK 36 374 and diltiazam. In: Prostaglandins and other eicosanoids in the cardiovascular system. Ed. K. Schror, Karger Basel

Gobel FL, Nordstrom LA, Nelson RR, Jorgenson CR, Wang Y (1978) The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 57: 549-556

Goldberg S, Greenspon AJ, Urban PL, Muza B, Berger B, Walinsky P, Maroko PR (1983) Reperfusion arrhythmia: A marker of restoration of antegrade flow during intracoronary thrombolysis for acute myocardial infarction. Am Heart J 105: 26

Goldstein S, Friedman L, Hutchinson R et al (1984) Timing, mechanism and clinical setting of witnessed deaths in post-myocardial infarction patients. J Am Coll Cardiol 3: 1111

Graeff PA de, Gilst WH van, Langen CDJ de, Wesseling H (1984) Concentration-dependent protection by captopril against reperfusion injury in the isolated rat heart. Circulation, 70, 4, II-89

Graeff PA de, Gilst WH van, Kingma JH, Langen CDJ de, Wesseling H (1986) Effects of captopril in a closed-chest pig model against ischemia-reperfusion injury. N-S Archiv Pharmacol, in press

Grinwald PM (1982) Calcium uptake during post-ischemic reperfusion in the isolated rat heart: influence of extracellular sodium. J Mol Cell Cardiol 14: 359-365

Grinwald PM, Nayler WG (1981) Calcium entry in the calcium paradox. J Mol Cell Cardiol 13: 867-880

Gross DM, Sweet CS, Ulm EH, Backlund EP, Morris AA, Weitz D, Bohn DL, WengerHC, Vassil TC, Stone CA (1980) Effect of N-(S)-1-carboxy-3-phenylpropyl-L-ala-Lpro and its ethyl ester (MK-421) on angiotensin converting enzyme in vitro and angiotensin I pressor responses in vivo. J Pharm Exp Ther 216: 552-558

Guarnieri C, Flamingni F, Caldarer CM (1980) Role of oxygen in the cellular damage induced by reoxygenation of hypoxic heart. J Mol Cell Cardiol 10: 893-906

Hamberg M, Svensson J, Samuelsson B (1975) Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 72: 2994-2998

Harmsen E, Jong JW de, Serruys PW (1981) Hypoxanthine production by ischemic heart demonstrated by high performance liquid chromatography of blood purine nucleosides and oxypurines. Clin Chim Acta 115: 73

Harmsen E (1984) Myocardial purine metablism. Aspects of myocardial ATP metabolsim and pharmacological intervention. Thesis, Rotterdam

Hearse DJ, Humphrey SM, Nayler WG, Slade A, Border D (1975) Ultrastructural damage associated with reoxygenation of the anoxic myocardium. J Mol Cell Cardiol 7: 314-324

Hearse DJ (1977) Reperfusion of the ischaemic myocardium. J Mol Cell Cardiol 9: 605-616

Hearse DJ, Garlick PB, Humphrey SM (1977) Ischemic contracture of the myocardium: Mechanism and prevention. Am J Cardiol 39: 986-993

Hearse DJ, Humphrey SM, Bullock GR (1978) The oxygen paradox and the calcium paradox: Two facets of the same problem. J Mol Cell Cardiol 10: 641-668

Hearse DJ (1979) Oxygen deprivation and early myocardial contractile failure. Reassessment of the possible role of adenosine triphosphate. Am J cardiol 44: 1115-1120

Hearse DJ, Baker JE, Humphrey SM (1980) Verapamil and the calcium paradox. J Mol Cell Cardiol 12: 733-739

Hearse DJ, Crome R, Yellon DM, Wyse R (1983) Metabolic and flow correlates of myocardial ischaemia. Cardiovasc Res 17: 452-458

Hearse DJ (1984) Critical distinctions in the modification of myocardial cell injury In: Opie LH (ed) Calcium Antagonists and cardiovascular disease. New York: Raven Press

Heimburger RF (1946) Injection into pericardial sac and ligation of coronary artery of the rat. Arch Surg 52: 677

Henry PD (1980) Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltizam. Am J Cardiol 46: 1047

Henry PD, Wahl AM (1983) Diltiazem and nitrendipine suppress hypoxic contracture in quiescent ventricular myocardium. Eur Heart J 4: 819-832

Hess ML, Warner MF, Robbins AD, Crute S, Greenfield LJ (1981a) Characterization of the excitation-contraction coupling system of the hypothermic myocardium following ischemia and reperfusion. Cardiovasc Res 15: 390-397

Hess ML, Okabe E, Kontos HA (1981b) Proton and free oxygen radicle interaction with the calcium transport system of cardiac sarcoplasmic reticulum. J Mol Cell Cardiol 13: 767-773

Hirsch PD, Hillis LD, Campbell WB, Firth BG, Willerson JT (1981) Release of prostaglandins and thromboxane into the coronary circulation in patients with ischaemic heart disease. N Eng J Med 304: 685-691

Hjalmarson A (1980) Myocardial metabolic changes related to ventricular fibrillation. Cardiology 65: 226-247

Hjalmarson A, Elmfeldt D, Herlitz J, Holmberg S, Malek I, Nyberg G, Ryden L, Swedberg K, Vedin A, Waagstein F, Waldenstrom A, Waldenstrom I, Wedel H, Wilhelmsen L, Wilhelmsson C (1981) Effect on mortality of metoprolol in acute myocardial infarction. Lancet 2: 823-827

Hock CE, Ribeiro LGT, Lefer AM (1985) Preservation of ischemic myocardium by a new converting enzyme inhibitor, enalaprilic acid, in acute myocardial infarction. Am Heart J 109: 222-228

Holland CE, Olson RE (1975) Prevention by hypothermia of paradoxical necrosis in cardiac muscle. J Mol Cell Cardiol 7: 917-928

Ichihara K, Abiko Y (1984) Rebound recovery of myocardial creatine phosphate with reperfusion after ischemia. Am Heart J 108: 1594-1597

Ideker RE, Klein GS, Harrison L, Smith WM, Kasell J, Reimer KA, Wallace AG, Gallagher JJ (1981) The transition to ventricular fibrillation induced by reperfusion after acute ischemia in the dog: a period of organized epicardial activation. Circulation 63: 1371-1379

Iseri LT, French JH (1984) Magnesium: Nature's physiologic calcium blocker. Am Heart J 108: 188-193

Janse MJ, Cinca J, Morena H, Fiolet JWT, Kleber AG, Vries GP de, Becker AE, Durrer D (1979) The "border zone" in myocardial ischemia. An electrophysiological, metabolic and histochemical correlation in the pig heart. Circ Res 44: 576-588

Janse MJ, Kleber AG (1981) Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischemia. Circ Res 49: 1069-1081

Jennings RB, Ganote CE, Reimer KA (1975) Ischemic tissue injury. Am J Pathol 81: 179-198

Jennings RB, Reimer KA (1981) Lethal myocardial ischemic injury. Am J Pathol 102: 241-255

Jennings RB (1984) Calcium ions in ischemia. In: Calcium antagonists and cardiovascular disease, edited by LH Opie. New York, Raven Press; 85-95

Jennings RB, Reimer KA, Steenbergen C (1984) Myocardial ischemia and reperfusion: role of calcium. In: Control and manipulation of calcium movement, ed. Parratt JR, Raven Press, New York

Johns NP, Olson BJ (1954) Experimental myocardial infarction I. A method of coronary occlusion in small animals. Annals Surg 140: 675

Johnston KM, Macleod BA, Walker MJA (1983) Responses to ligation of a coronary artery in conscious rats and the actions of antiarrhythmics. Can J Physiol Pharmacol 61: 1340-1353

Jolly SR, Menahahan LA, Gross GJ (1981) Diltiazem in myocardial recovery from global ischemia and reperfusion. J Mol Cell Cardiol 13: 359-372

Jong JW de, Verdouw PD, Remme WJ (1977) Myocardial nucleoside and carbohydrate metabolism and hemodynamics during partial occlusion and reperfusion of pig coronary artery. J Mol Cell Cardiol 9: 297

Jong JW de, Harmsen E, Tombe PP de, Keyzer E (1983) Release of purine nucleosides and oxypurines from the isolated perfused rat heart. Adv Myocardiol 4: 339-345

Jong JW de, Harmsen E, Tombe PP de (1984) Diltiazem administered before or during myocardial ischemia decreases adenine nucleotide catabolism. J Mol Cell Cardiol 16: 363-370

Jorgensen L, Rowsell HC, Hovigt T, Glynn MF, Mustard JF (1967) Adenosine diphosphate-induced platelet aggregation and myocardial infarction in swine. Lab invest 17: 616

Jugdutt BI, Hutchins GM, Bulkley BH, Becker LC (1981) Dissimilar effects of prostacyclin, prostaglandin E1, and prostaglandin E2 on myocardial infarct size after coronary occlusion in conscious dogs. Circ Res 49: 685-700

Julicher RHM, Tijburg LBM, Sterrenberg L, Bast A, Koomen JM, Noordhoek J (1984) Decreased defence against free radicals in rat heart during normal reperfusion after hypoxic, ischemic and calcium-free perfusion. Life Sciences 35: 1281-1288

Kane KA, McDonald FM, Parratt JR, Timmer C, Vink J (1982) Antiarrhythmic effects of Org 6001 in rats: correlation with plasma and tissue drug concentrations. Br J Pharmacol 72: 512-513

Kane KA, Parratt JR, Williams FM (1984) An investigation into the characteristics of reperfusion-induced arrhythmias in the anaesthetized rat and their susceptibility to antiarrhythmic agents. Br J Pharmac 82: 349-357

Kannengieser GJ, Lubbe WF, Opie LH (1975) Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. J Mol Cell Cardiol 7: 135- 151

Kaplinsky ES, Ogawa S, Michelson EL, Dreifus LS (1981) Instanteneous and delayed ventricular arrhythmias after reperfusion of acutely ischemic myocardium: evidence for multiple mechanisms. Circulation 63: 333-340

Karlinger JS, Motulsky HJ, Dunlap J, Brown JH, Insel PA. Verapamil competitively inhibits alpha1-adrenergic and muscarinic but not beta-adrenergic receptors in rat myocardium. J Cardiovasc Pharmacol 4: 515-522

Karlsberg RP, Henry PD, Ahmed SA, Sobel BE, Roberts R (1977) Lack of protection of ischemic myocardium by verapamil in conscious dogs. Eur J Pharmacol 42: 339

Karlsberg RP, Cryer PE, Roberts R (1981) Serial plasma catecholamine respons early in the course of clinical acute myocardial infarction: Relationship to infarct extent and mortality. Am Heart J 102: 24

Kass RS, Tsien RW (1975) Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibres. J Gen Physiol 66: 169-192

Kass RS, Lederer WJ, Tsien RW, Weingart R (1978) Role of calcium ions in transient inward current and after contraction induced by strophanthidin in cardiac Purkinje fibers. J Physiol 281: 187

Katz AM, Reuter H (1979) Cellular calcium and cardiac cell death. Am J Cardiol 44: 188-190

Kerin NZ, Rubenfire M, Willens HJ, Rao P, Cascade PN (1983) The mechanism of dysrhythmias in variant angina pectoris: Occlusive versus reperfusion. Am Heart J 106: 1332

Khan MT, Malik KU (1982) Modulation by prostaglandins of the release of H3noradrenaline evoked by potassium and nerve stimulation in the isolated rat heart. Eur J Pharmacol 78: 213-218

Kimura E, Kishida H (1981) Treatment of variant angina with drugs: a survey of 11 cardiology institutes in Japan. Circulation 63L: 844

Kleber AG (1984) Extracellular potassium accumulation in acute myocardial ischemia. J Mol Cell Cardiol 16: 389-394

Klein HH, Schubothe M, Nebendahl K, Kreuzer H (1984) The effect of two different diltiazem treatments on infarct size in ischemic, reperfused porcine hearts. Circulation 69: 1000-1005

Klein HH, Schubothe M, Nebendahl K, Kreuzer H (1984) Temporal and spatial development of myocardial infarcts in porcine hearts without significant collateral blood flow. Texas Heart Inst J 11: 154-159

Kloner RA, Ganote CE, Reimer KA, Jennings RB (1975) Distribution of coronary arterial flow in acute myocardial ischemia. Arch Pathol 99: 86-94

Kloner RA, Fishbein MC, Maclean D, Braunwald E, Maroko PR (1977) Effect of hyaluronidase during the early phase of acute myocardial ischemia: an ultrastructural and morphometric analysis. Am J Cardiol 40: 43

Kloner RA, Braunwald E (1980) Observations on experimental myocardial ischemia. Cardiovasc Res 14: 371-395

Kloner RA, Boer LWV de, Dargie JR, Ingwall JS, Hale S, Tumas J, Braunwald E (1981) Prolonged abnormalities of myocardium salvaged by reperfusion. Am J Physiol 241: H591-H599

Koomen JM, Jager LP, Noordwijk J van (1980) Effects of perfusion pressure on coronary flow, myocardial Ca;washout, and the occurence of calcium paradox in isolated perfused rat heart. Bas Res Cardiol 75: 318-327

Koomen JM, Gilst WH van, Zimmerman ANE, Noordwijk J van (1982) A concentration-dependent biphasic positive inotropic action of ouabain on isolated hearts of rat and guinea-pig. Arch Int Pharmacodyn Ther 255: 2

Koomen JM, Schevers JAM, Noordhoek J (1983) Myocardial recovery from global ischemia and reperfusion: Effects of pre- and/or postischemic perfusion with low-Ca. J Mol Cell Cardiol 15: 383-392

Kostyuk PG, Krishtal OA, Pidoplichko VI (1981) Calcium inward current and related charge movements in the membrane of snail neurones. J Physiol 310: 403-421

Krishtal OA, Pidoplichko VI, Shakhovalov YA (1981) Conductance of the calcium channel in the membrane of snail neurones. J Physiol 310: 423-434

Kraemer RS, Phernetton TM, Folts JD (1976) Prostaglandin-like substances in coronary venous blood following myocardial ischaemia. J Pharmac Exp Ther 199: 611-619

Kugler G (1979) Myocardial release of lactate, inosine and hypoxanthine during atrial pacing and excercise-induced angina. Circulation 59: 43-49

Labrid C, Grosset A, Dareng G, Mironneau J, Duchene-Marullaz P (1979) Some membrane interactions with bepridil, a new antianginal agent. J Pharmacol Exp Ther 211: 546-554

Langen CDJ de, Gilst WH van, Wesseling H (1984) Sustained protection by iloprost of the porcine heart in the acute and chronic phases of myocardial infarction. Circulation 70: II-86

Langen CDJ de, Gilst WH van, Wesseling H (1985) Sustained protection by iloprost of the porcine heart in the acute and chronic phases of myocardial infarction. J Cardiovasc Pharmacol 7: 924-928

Langendorff O (1895) Untersuchungen am uberlebenden Saugetierherzen. Pfluegers Arch 61: 291

Lee Y, Chang S (1977) Effect of lysolecithin on the structure and permeability of lecithin bilayer vesicles. Biochem 16: 1303-1309

Lefer AM, Ogletree ML, Smith JB, Silver MJ, Nicolaou KC, Barnette WE, Gasic GP (1978) Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. Scinece 200: 52-54

Lefer AM, Araki H, Okamatsu S (1981) Beneficial actions of a free radical scavenger in traumatic shock and myocardial ischemia. Circ Shock 8: 273-282

Lefer AM, Peck RC (1984) Cardioprotective effects of enalapril in acute myocardial ischemia. Pharmacology 29: 61

Leiris J de, Harding DP, Pestre S (1984) The isolated perfused rat heart: a model for studying myocardial hypoxia or ischaemia. Basic Res Cardiol 79: 313-321

Levites R, Banka VS, Helfont RH (1975) Electrophysiologic effects of coronary occlusion and reperfusion: observations of dispersion of refractoriness and ventricular automaticity. Circulation 52: 760-765

Lewy RI, Smith JB, Silver MJ, Saia J, Walinsky P, Weiner L (1979) Detection of thromboxane B2 in peripheral blood of patients with Prinzmetal's angina. Prostaglandins Med 2: 243-248

Liang C, Gavras H, Black J, Sherman LG, Hood WB (1982) Renin-angiotensin system inhibition in acute myocardial infarction in dogs. Circulation 66: 1249-1255

Lowe JE, Reimer KA, Jennings RB (1978) Experimental infarct size as a function of the amount of myocardium at risk. Am J Pathol 90: 363

Lubbe WF, Daries PS, Opie LH (1978) Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc Res 12: 212-220

Lumb GD, Singletary HP (1962) Blood supply to the atrio-ventricular node and the bundle of His: a comparitive study in pig, dog and man. Am J Pathol 41: 65-75

MacAlpin RN (1980) Contribution of dynamic vascular wall thickening to luminal narrowing during coronary arterial constriction. Circulation 60: 296-301

Maclean D, Fishbein MC, Braunwald E, Maroko PR (1978) Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. J Clin Invest 61: 541-551

Macleod BA, Augereau P, Walker MJA (1983) Effects of halothane anaesthesia compared with fentanyl anaesthesia and no anaesthesia during coronary ligation in rats. Anesthesiology 58: 44-52

Manning AS, Hearse DJ, Dennis SC, Bullock GR, Coltart DJ (1980) Myocardial ischaemia: an isolated, globally perfused rat heart model for metabolic and pharmacological studies. Eur J Cardiol 11: 1-21

Manning AS, Crome R, Isted K, Coltart DJ, Hearse DJ (1983) Pharmacological prevention of reperfusion-induced ventricular fibrillation in the isolated rat heart. J Mol Cell Cardiol 15 (suppl 1): 413

Manning AS, Coltart DJ, Hearse DJ (1984) Ischemia and reperfusion-induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with alopurinol. Circ Res 55: 545-548

Manning AS, Hearse DJ (1984) Reperfusion-induced arrhythmias: mechanisms and prevention. J Mol Cell Cardiol 16: 497-518

Marcus ML, Kerber RE, Ehrhardt J, Abboud FM (1976) Effects of time on volume and distribution of coronary collateral flow. Am J Physiol 230: 279

Markis JE, Malagold M, Parker JA, Silverman KJ, Barry WH, Als AV, Paulen S, Grossman W, Braunwald E (1981) Myocardial salvage after intracoronary thrombolysis with streptokinase in acute myocardial infarction, assessment with intracoronary thallium-201. N Eng J Med 305: 777

Maroko PR, Kjekshus JK, Sobel BE, Watanabe T, Covell JW, Ross J, Braunwald E (1971) Factors influencing infarct size following experimental coronary artery occlusions. Circulation 43: 67- 82

Maseri A, L'Abbate A, Pesola A, Ballestra AM, Marzilli M, Severi S, Maltinti G, DeNes DM, Parodi O, Biagini A (1977a) Coronary vasospasm in angina pectoris. Lancet 1: 713

Maseri A, Severi S, L'Abbate A, Pesola A (1977b) "Variant" angina: one aspect of a continuous spectrum of vasospastic angina. Circulation 55-56 (suppl. III): III-120

Maseri A, L'Abbate A, Chierchia S, Parodi O, Severi S, Biagini A, Distante A, Marzilli, M, Ballestra AM (1979) Significance of spasm in the pathogenesis of ischemic heart disease. Am J Cardiol 44: 788-792

Maseri A (1980) Pathogenetic mechanisms of angina pectoris: expanding views. Br Heart J 43: 648-660

Maseri A, Chierchia S, Kaski JC (1985) Mixed angina pectoris. Am J Cardiol 56: 30E-33E

Marshall RJ, Muir AW, Winslow E (1981) Development of a severe model of early coronary artery ligation-induced dysrhythmias in the anaesthetized rat. Br J Pharmacol 73: 951-959

Mathey DG, Kuck KH, Tilsner V, Krebber HJ, Bleifeld W (1981) Nonsurgical coronary artery recanalization in acute transmural myocardial infarction. Circulation 63: 489

McCord JM, Fridovich I (1968) The reduction of cytochrome C by milk xanthine oxidase. J Biol Chem 243: 5753-5760

McCord JM (1985) Oxygen-derived free radicals in postischemic tissue injury. N Eng J Med 312: 159-163

Meerson FZ, Ustinova EE (1982) Prevention of stress damage to the heart and its hypoxic contraction with the natural antioxidant alpha-tocopherol. Kardiologiya 22: 89-94

Melin JA, Becker LC (1983) Salvage of ischemic myocardium by prostacyclin during experimental myocardial infarction. J Am Coll Cardiol 2: 279-286

Menken U, Wiegand V, Bucher P, Meesmann W (1979) Prophylaxis of ventricular fibrillation after acute experimental occlusion by chronic beta-adrenoceptor blockade with atenolol. Cardiovasc Res 13: 588-594

Miller DP, Waters DD, Szlachcic J, Theroux P (1982) Clinical characteristics associated with sudden death in patients with variant angina. Circulation 66: 588-592

Mullane KM, Moncada S (1980) Prostacyclin mediates the potentiated hypotensive effect of bradykinin following captopril treatment. Eur J Pharmacol 66: 355-365

Murdock DK, Loeb JM, Euler DE, Randall WC (1980) Electrophysiology of coronary reperfusion, a mechanism for reperfusion arrhytymias. Circulation 61: 175-182

Naimi S, Avitall B, Meiszala J, Levine HJ (1977) Dispersion of effective refractory period during abrupt reperfusion of ischemic myocardium in dogs. Am J Cardiol 39: 407-412

Naito M, Michelson E, Metzo K, Kaplinsky E, Dreifus L (1981) Failure of anti-arrhythmic drugs to prevent experimental reperfusion ventricular fibrillation. Circulation 63: 70-79

Nakajima H, Hoshiyama M, Yamashita K, Kiyomoto A (1975) Effect of diltiazem on electrical and mechanical activity of isolated cardiac ventricular muscle of guinea pig. Jpn J Pharmacol 25: 383-392

Nanda V, Henry PD (1982) Increased serotonergic and alpha adrenergic receptors in aortas from rabbits fed a high cholesterol diet. Clin Res 30: 209A

Nayler WG (1980a) Calcium antagonists. Eur Heart J 1: 225-237

Nayler WG (1980b) Cardioprotective effects of calcium ion antagonists in myocardial ischemia. Clin Invest Med 3: 91-99

Nayler WG, Ferrari R, Williams A (1980) Protective effect of pretreatment with verapamil, nifedipine and propranolol on mitochondrial function in the ischemic and reperfused myocardium. Am J Cardiol 46: 242

Nayler WG (1981) The role of calcium in the ischemic myocardium. Am J Pathol 102: 262-270

Nayler WG (1982) Protection of the myocardium against post-ischemic reperfusion damage. J Thorac Cardiovasc Surg 84: 897-905

Nayler WG, Thompson JE, Jarrott B (1982) The interaction of calcium antagonists (slow channel blockers) with myocardial alpha adrenoceptors. J Mol Cell Cardiol 14: 185-188

Nayler WG, Sturrock WJ (1983) An inhibitory effect of verapamil and diltiazem on the release of noradrenaline from ischaemic and reperfused hearts. J Mol Cell Cardiol 16: 331-344

Nayler WG, Purchase M, Dusting GJ (1984) Effect of prostacyclin infusion during low-flow ischaemia in the isolated perfused rat heart. Basic Res Cardiol 79: 125-134

Nayler WG, Sturrock WJ (1985) Inhibitory effect of calcium antagonists on the depletion of cardiac norepinephrine during postischemic reperfusion. J Cardiovasc Pharmacol 7: 581-587

Nayler WG, Sturrock WJ, Panagiotopoulos S (1985) Calcium and myocardial ischemia In: Control and manipulation of calcium movement, ed JR Parratt, Raven Press New York, 303-324

Neely JR, Rovetto MJ, Whitmer JT, Morgan HE (1973) Effects of ischemia on function and metabolism of the isolated working rat heart. Am J Physiol 225: 651-658

Neely JR, Liedtke AJ, Whitmer JT, Rovetto MJ (1975) Relationship between coronary flow and adenosine triphosphate production from glycolysis and oxidative metabolism. In: "The cardiac sarcoplasm", Eds. Roy HE, Harris P, Univ Park Press, Baltimore. 8: 301-321

Neely JR, Whitmer KM, Mochizuki S (1976) Effects of mechanical activity and hormones on myocardial glucose and fatty acid utilization. Circ Res 38 (I): 22 - 30

Ogletree ML, Flynn JT, Feola M, Lefer AM (1977) Early prostaglandin release from ischaemic myocardium in the dog. Sarg Gynecol Obstetr 144: 734-740

Ogletree ML, Lefer AM, Smith JB, Nicolaou KC (1979) Studies on protective effect of prostacyclin in acute myocardial ischemia. Eur J Pharmacol 56: 95-103

Oliva PB, Potts DE, Pluss RG (1973) Coronary arterial spasm in Prinzmetal angina: documentation by coronary arteriography. N Eng J Med 288: 745

Oliva PB, Breckenridge JC (1977) Arteriographic evidence of coronary arterial apasm in acute myocardial infarction. Circulation 56: 366-374

Olsson RA (1970) Changes in content of purine nucleoside in canine myocardium during coronary occlusions. Circ Res 26: 301-306

Ong L, Reiser P, Coromilas J, Scherr L, Morrison J (1983) Left ventricular function and rapid release of creatine kinase MB in acute myocardial infarction. Evidence for spontaneous reperfusion. N Engl J Med 309: 1-6

Opie LH, Bruyneel KJJ, Owen P (1975) Beneficial effects of glucose, potassium and insulin infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. Circulation 52: 49-57

Opie LH (1976) Effects of regional ischemia on metabolism of glucose and fatty acids. Relative rates of aerobic and anaerobic energy production during myocardial infarction. Circ Res 38 (suppl 1): 52-74

Opie LH, Nathan D, Lubbe WF (1979) Biochemical aspects of arrhythmogenesis and ventricular fibrillation. Am J Cardiol 43: 131-148

Opie LH (1980) Calcium antagonists. Lancet 13983 (1): 806

Opie LH (1982) Role of cyclic nucleotides in heart metabolism. Cardiovasc Res 16: 483-507

Opie LH, Bruyneel KJJ, Lubbe WF (1983) What has the baboon to offer as a model of experimental ischemia? Eur Heart J 4 (Suppl. C): 55-60

Opie LH (1984) Calcium antagonists. Mechanisms, therapeutic indications and reservations: a review. Quart J Med 209: 1

Opie LH, Thandroyen F (1984) Molecular and biochemical mechanisms underlying the role of calcium ions in malignant ventricular arrhythmias. Annual NY Acad Sci 427: 127-139

Parratt JR, Coker SJ (1985) Thromboxane, prostacyclin, and the severity of early ischemic and reperfusion arrhythmias ; a review of the evidence. In: Prostaglandins and other eicosanoids in the cardiovascular system. Ed. K. Schror, Karger Basel, 172-182.

Penkoske PA, Sobel BE, Corr PB (1978) Disparate electrophysiological alterations accompanying dysrhythmia due to coronary occlusion and reperfusion in the cat. Circulation 58: 1023-1035

Penny WJ, Sheridan DJ (1983) Arrhythmias and cellular electrophysiological changes during myocardial "ischaemia" and reperfusion. Cardiovasc Res 17: 363-272

Penny WJ (1984) The deleterious effects of myocardial catecholamines on cellular electrophysiology and arrhythmias during ischaemia and reperfusion. Eur Heart J 5: 960-973

Perez JE, Sobel BE, Henry PD (1980) Improved performance of ischemic canine myocardium in response to nifedipine and diltiazem. Am J Physiol 239: H658-H668

Peter T, Norris RM, Clarke ED, Heng MK, Singh BN, Williams B, Howell DR, Ambler PK (1978) Reduction of enzyme levels by propranolol after acute myocardial infarction. Circulation 57: 1091-1095

Plageman PGW, Wohlhueter RM (1983) Nucleoside transport in mammalian cells and interaction with intracellular metabolism. In: Berne RM, Randle TW, Rubio R eds. Regulatory function of adenosine. Nijhoff Publs, The Hague. 179-201

Podzuweit T, Dalby AJ, Cherry GW, Opie LH (1978) Cyclic AMP levels in ischaemic and non-ischaemic myocardium following coronary artery ligation: relation to ventricular fibrillation. J Mol Cell Cardiol 10: 81-94

Previtali M, Klersy C, Salerno JA, Chimienti M, Panciroli C, Marangoni E, Specchia G, Comolli M, Bobba P (1983) Ventricular tachyarrhythmias in Prinzmetal's variant angina: Clinical significance and relation to the degree and time course of S-T segment elevation. Am J Cardiol 52: 19-25

Putney JW, Weiss JJ, van de Walle CM, Haddas RA (1980) Is phosphatidic acid a calcium inophore under neurohumoral control. Nature 284: 345-347

Rao PS, Cohen MV, Mueller HS (1983) Production of free radicals and lipid peroxides in early experimental myocardial ischemia. J Mol Cell Cardiol 15: 713-716

Reimer KA, Rasmussen MM, Jennings RB (1976) On the nature of protection by propranolol against myocardial necrosis after temporary occlusion in dogs. Am J Cardiol 37: 520-527

Reimer KA, Lowe JE, Jennings RB (1977) Effect of the calcium antagonist verapamil on necrosis following temporary coronary artery occlusion in dogs. Circulation 55: 581

Reimer KA, Hill ML, Jennings RB (1981) Prolonged depletion of ATP and of adenine nucleotide pool, due to delayed resynthesis of adenine nucleotides following reversible myocardial ischemic injury in dogs. J Mol Cell cardiol 13: 229-239

Rentrop P, Blanke H, Karsch KR, Kaiser H, Kostering H, Leitz K (1981) Selective intracoronary thrombolysis in acute myocardial infarction and unstable angina pectoris. Circulation 63: 307

Reuter H (1974) Exchange of calcium ions in the mammalian myocardium. Circ Res 34: 599-605

Ribeiro LGT, Brandon TA, Debauch TL, Maroko PR, Miller RR (1981) Anti-arrhythmic and hemodynamic effects of calcium channel blocking agents during cornary arterial reperfusion. Am J Cardiol 48: 69-74

Rinaldi ML, Capony JP, Demaille JG (1982) The cyclic AMP-dependent modulation of cardiac sarcolemmel slow channels. J Mol Cell Cardiol 14: 279-289

Robertson RM, Robertson D, Roberts LJ, Maas RL, Fitzgerald GA, Friesinger GC, Oates JA (1981) Thromboxane A2 in vasotonic angina pectoris: Evidence from direct measurement and inhibitor trials. N Eng J Med 304: 998-1003

Rochette L, Didier JP, Moreau D, Bralet J, Opie LH (1984) Role of beta-adrenoceptor antagonism in the prevention of reperfusion ventricular arrhythmias: Effects of acebutolol, atenolol, and d-propranolol on isolated working rat hearts subject to myocardial ischemia and reperfusion. Am Heart J 107: 1132-1141

Rousseau MF, Bertrand ME, Detry JMR, Decoster PM,

Lablanche JM (1982) Coronary collaterals and left ventricular function early after acute transmural myocardial infarction. Eur Heart J 3: 223

Rovetto MJ, Lamberton WF, Neely JR (1975) Mechanisms of glycolytic inhibition in ischemic rat hearts. Circ Res 37: 742-751

Russell DC, Wojtczak J, Oliver MF (1977) Combined electrophysiological technique for assessment of the cellular basis of early ventricular arrhythmias. Lancet 2: 686-688

Russell DC, Oliver MF (1978) Ventricular refractoriness during acute myocardial ischaemia and its relationship to ventricular fibrillation. Cardiovasc Res 12: 221-227

Sakai K, Gebhard MM, Spieckermann PG, Bretschneider HJ (1975) Enzyme release resulting from total ischemia and reperfusion in the isolated, perfused guinea pig heart. J Mol Cell Cardiol 7: 827-840

Saruta T, Suzuki H, Okundo T, Kondo K (1982) Effects of angiotensin-converting enzyme inhibitors on the vascular response to norepinephrine. Am J Cardiol 49: 1535-1536

Schaal SF, Wallace AG, Sealy WC (1969) Protective influence of cardiac denervation against arrhythmias of myocardial infarction. Cardiovasc Res 3: 241-244

Schaper W, Jageneau A, Xhonneux R (1967) The development of collateral circulation in the pig and dog heart. Cardiologia 51: 321-335

Schaper W (1979) The collateral circulation of the heart. Amsterdam: Elsevier/North Holland

Schaper J, Mulch J, Winkler B, Schaper W (1979) Ultrastructural, functional, and biochemical criteria for estimation of reversibility of ischemic injury: a study on the effects of global ischemia on the isolated dog heart. J Mol Cell Cardiol 11: 521-541

Schömig A, Dart AM, Dietz R, Mayer E, Kubler W (1984) Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: Locally mediated release. Circ Res 55: 689-701

Schrör K, Ohlendorf R, Darius H (1981) Beneficial effects of a new carbacyclin derivative, ZK 36 374, in acute myocardial ischemia. J Pharmacol Exp Therap 219: 243-249

Schrör K, Darius H, Addicks K, Koster R, Smith EF (1982) PGI2 prevents ischemia-induced alterations in cardiac catecholamines without influencing nerve-stimulation-induced catecholamine release in non-ischemic conditions. J Cardiovasc Pharmacol 4: 741-748

Schrör K, Funke K (1985) Prostaglandins and myocardial noradrenaline overflow after sympathetic nerve stimulation during ischemia and reperfusion. J Cardiovasc Pharmacol 7 (suppl. 5): S50-S54

Schwartz CJ, Gerrity RS (1975) Anatomical pathology of sudden unexpected cardiac death. Circulation 52: III-18

Schwartz PJ, Stone HL (1980) Left stellectomy in the prevention of ventricular fibrillation caused by acute myocardial ischemia in conscious dogs with anterior infarction. Circulation 62: 1256-1265

Sedlis SP, Corr PB, Sobel BE, Ahumada GG (1983) Lysophosphatidyl choline potentiates calcium accumulation in rat cardiac myocytes. Am J Physiol 244: H32-H38

Selye H, Bajusz E, Grasso S, Medell P (1960) Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11: 398

Serneri GGN, Gensini GF, Abbate R, Prisco D, Rogasi PG, Laureano R, Casolo GC, Fantini F, Donato M, Dabizzi RP (1985) Abnormal cardiocoronary thromboxane A2 production in patients with unstable angina. Am Heart J 109: 732-739

Sharma A, Lee B, Saffitz B, Sobel BE, Corr PB (1983) Alpha adrenergic mediated accumulation of calcium in reperfused myocardium. J Clin Invest 72: 802-818

Sheehan FH, Epstein SE (1982) Determinants of arrhythmic death due to coronary spasm: effect of pre-existing coronary artery stenosis on incidence of reperfusion arrhythmia. Circulation 65: 259-264

Shen AC, Jennings RB (1972) Kinetics of calcium accumulation in acute ischemic myocardial injury. Am J Pathol 67: 417-440

Sheridan DJ, Penkoske PA, Sobel BE, Corr PB (1980) Alpha-adrenergic contributions to dysrhythmia during myocardial ischaemia and reperfusion in cats. J Clin Invest 65: 161-171

Shine KI, Douglas AM, Ricchiuti N (1978) Calcium, strontium, and barium movements during ischemia and reperfusion in rabbit ventricle. Circ Res 43: 712-720

Shlafer M, Kane PF, Wiggins VY, Kirsh MM (1982) Possible role for cytotoxic oxygen metabolites in the pathogenesis of cardiac ischemic injury. Circulation 66: I82-I92

Smith GT, Geary G, Ruf W, Roelofs TH, McNamara JJ (1979) Epicardial mapping and electrocardiographic models of myocardial ischemic injury. Circulation 60: 930-938

Smith EF, Kloster G, Stocklin K, Schrör K (1984) Effect of iloprost (ZK 36 374) on membrane integrity in ischemic rabbit hearts. Biomed Biochem Acta 43:155-158

Sperelakis N, Schneider JA (1976) A metabolic control mechanism for calcium influx that may protect the ventricular myocardial cell. Am J Cardiol 37: 1079-1085

Sperelakis N (1984) Properties of calcium-dependent slow action potentials: Their possible role in arrhythmias. In: Calcium antagonists and cadiovascular disease. Ed. Opie LH, Raven Press, New York, 277-291

Stam H, Jong JW de (1977) Sephadex-induced reduction of coronary flow in the isolated rat heart: a model for ischemic heart disease. J Mol Cell Cardiol 9: 633

Stewart JR, Burmeister WE, Burmeister J, Lucchesi BR (1980) Electrophysiologic and antiarrhythmic effects of phentolamine in experimental coronary occlusion and reperfusion in the dog. J Cardiovasc Pharmacol 2: 77-91

Sugiyama S, Ozawa T, Kato T, Suzuki S (1980a) Recovery time course of ventricular vulnerability after coronary reperfusion in relation to mitochondrial function in ischemic myocardium. Am Heart J 100: 829-837

Sugiyama S, Ozawa T, Suzuki S, Kato T (1980b) Effects of verapamil and propranolol on ventricular vulnerability after coronary reperfusion. J Electrocardiol 13: 49-54

Swartz SL, Williams GH, Hollenberg NK, Levine L, Dluhy RG, Moore, FJ (1980) Captopril-induced changes in prostaglandin production. J Clin Invest 65: 1257

Swenne CA, Van Hemel NM (1983) Algorithms for the interpretation of ventricular arrhythmias In: Proc Computers in Cardiol Silver Spring, MD, IEEE Computer Society Press 231-241

Tada M, Kuzuya T, Inove M, Kodama K, Mishima M, Inui M, Abe H (1981) Elevation of thromboxane B2 levels in patients with classic and variant angina pectoris. Circulation 64: 1107-1115

Tennant R, Wiggers CJ (1935) The effect of coronary occlusion on myocardial contraction. Am J Physiol 112: 351-361

Thandroyen FT, Worthington MG, Higginson LM, Opie LH (1983) The effect of alpha- and beta-adrenoceptor antagonist agents on reperfusion ventricular fibrillation and metabolic status in the isolated perfused ratheart. J Am Coll Cardiol 1: 1056-1066

Thompson CI, Rubio R, Berne RM (1980) Changes in adenosine and glycogen phosphorylase activity during the cardiac cycle. Am J Physiol 238: H389-H398

Tones MA, Poole-Wilson PA (1985) Alpha-adrenoceptor stimulation, lysophosphoglycerides, and lipid peroxidation in reoxygenation induced calcium uptake in rabbit myocardium. Cardiovasc Res 19: 228-236

Tzivoni D, Keren A, Granot H, Gottlieb S, Benhorin J, Stern S (1983) Ventricular fibrillation caused by myocardial reperfusion in Prinzmetal's angina. Am Heart J 105: 323-325

Verdouw PD, Hartog JM, ten Cate FJ, Schamhardt HC, Bastiaans OL, van Bremen RH, Serruys PW, Hugenholtz PG (1981) Effects of nifedipine on the recovery of regional myocardial performance during reperfusion of ischaemic myocardium. Progr Pharmacol 4: 91-100

Verdouw PD, Wolffenbuttel BHR, Giessen WJ van der (1983) Domestic pigs in the study of myocardial ischemia. Eur Heart J 4 (Suppl. C): 61-67

Voudoukis IJ (1970) Exaggerated cold-pressor response in hypertensive patients with superimposed arteriosclerosis. J Surg Oncol 2: 83-87

Wakade AR, Furchgott RF (1968) Metabolic requirements for the uptake and storage of norepinephrine by the isolated left atrium of the guinea-pig. J Pharmacol Exp Ther 163: 123-135

Walinsky P, Smith JB, Lefer AM, Lebenthal M, Urban P, Greenspon A (1984) Thromboxane A2 in acute myocardial infarction. Am Heart J 108: 868-872

Weishaar R, Ashikawa K, Bing RJ (1979) Effect of diltiazem, a calcium antagonist, on myocardial ischemia. Am J Cardiol 43: 1137-1143

Weishaar RE, Bing RJ (1980) The beneficial effect of a calcium channel blocker, diltiazem, on the ischemic-reperfused heart. J Mol Cell Cardiol 12: 993-1009

Weiss J, Shine KI (1982) K accumulation and electrophysiological alterations during early myocardial ischemia. Am J Physiol 243: H318-H327

Westerink BHC (1983) Analysis of trace amounts of catecholamines and related compounds in brain tissue: a study near the detection limit of liquid chromatography with electrochemical detection. J Liquid Chrom 6, 12: 2337-2351

Whalen DA, Hamilton DG, Ganote CE, Jennings RB (1974) Effect of a transient period of ischemia on myocardial cells. I. Effects on cell volume regulation. Am J Pathol 74: 381-398

White FC, Bloor CM (1981) Coronary collateral circulation in the pig: correlation of collateral flow with coronary bed size. Basic Res Cardiol 76: 189

Wilson DF, Erecinska M, Brown C, Silver IA (1977) Effect of oxygen tension on cellular energetics. Am J Physiol 233: C135-C140

Winslow E, Marshall RJ, Hope FG (1983) Comparative effects of fast- and slow-channel blocking agents on reperfusion-induced arrhythmias in the isolated perfused rat heart. J Cardiovasc Pharmacol 5: 928-936

Witzgall H, Hirsch F, Scherer B, Weber PC (1982) Acute haemodynamic and hormonal effects of captopril are diminished by indomethacin. Clin Sci 62: 611

Woodward B, Zakaria M (1985) Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J Mol Cell Cardiol 17: 485-493

Yasminek VG, Pyle RB, Nicoloff DM (1976) Rate of decay and distribution volume of MB isoenzyme of creatine kinase, intravenously injected in the baboon. Clin Chem 22: 1095-1097

Yellon DM, Hearse DJ, Maxwell MP, Chambers DE, Downey JM (1983) Sustained limitation of myocardial necrosis 24 hours after coronary artery occlusion: verapamil infusion in dogs with small myocardial infarcts. Am J Cardiol 51: 1409

Yusuf S, Ramsdale D, Peto R, Furse L, Bennett D, Bray C, Sleight P (1980) Early intravenous atenolol treatment in suspected acute myocardial infarction. Lancet 2: 273-276

Zimmerman ANE, Hulsmann WC (1967) Paradoxical influence of calcium ions on the permeability of the cell membranes of the isolated rat heart. Nature 211: 646-647

Zuanetti G, Vanoli E, Zaza A, Priori S, Stramba-Badiali M, Schwartz PJ (1985) Lack of correlation between occlusion and reperfusion arrhythmias in the cat. Am Heart J 109: 932-936

APPENDIX 1

IMPROVED FUNCTIONAL RECOVERY OF THE ISOLATED RAT HEART AFTER 24 HOURS OF HYPOTHERMIC ARREST WITH A STABLE PROSTACYCLIN ANALOGUE (ZK 36 374)

W.H. van Gilst, P.W. Boonstra, J.A. Terpstra,
Ch.R.H. Wildevuur and C.D.J. de Langen.

J Mol Cell Cardiol 15: 789-792, 1983

IMPROVED FUNCTIONAL RECOVERY OF THE ISOLATED RAT HEART AFTER 24 HOURS OF HYPOTHERMIC ARREST WITH A STABLE PROSTACYCLIN ANALOGUE (ZK 36 374)

Prostacyclin (PGI_2) can protect the heart against ischemia, i.e. it can reduce myocardial damage (9,10). PGI_2 protects the myocardium in vivo by preventing platelets from clumping and by dispersing preformed platelet aggregates (1,14). However, also in the absence of platelets, PGI_2 was shown to protect the myocardium against ischemia at concentrations that did not affect smooth muscle tone in the vessel wall (2). This protective effect of PGI_2 in vitro might be related to a stabilization of cell membranes in adrenergic nerve endings and hence to the prevention of ischemia-induced catecholamine release (13).

The instability of PGI_2, both in vitro and in vivo, limits its application during long ischemic periods. Recently, a stable prostacyclin analogue, ZK 36 374, was demonstrated to have several prostacyclin-mimetic activities, both in vitro and in vivo (11,12).

In this communication we report upon the beneficial effect of this stable prostacyclin analogue at a low concentration (4 nM) on the extent of ischemic damage, on the recovery of myocardial function and on the occurrence of arrhythmias in the isolated rat heart after 24 h hypothermic cardiac arrest.

Male Wistar rats (275-325 g) were anesthetized with diethyl-ether and heparinized intravenously. After excision, the hearts were subjected to Langendorff non-recirculating perfusion and were allowed to beat sponteneously. The perfusate was a modified Meyler bicarbonate buffer (pH 7.4) containing glucose (8) and was gassed at 37°C with 95% O_2 and 5% CO_2. This perfusion solution was filtered through 1.2 μm pore size filters before reaching the heart. The perfusion pressure was maintained at 60 mmHg.

After equilibration, the hearts were perfused for a control period of 15 min. Thereafter cardiac arrest was effected by perfusion of the heart with St Thomas Hospital solution (4 ml/min, temp. 10°C) (6) for a period of 2 min. Cardiac arrest was complete within 1 min of this perfusion. Subsequently, the hearts were stored for 24 h in 20 ml St Thomas Hospital solution, which was continuously gassed with 95% O_2 and 5% CO_2, while its temperature was kept at 10°C. Reperfusion of the hearts after this period of 24 h was performed with the modified Meyler perfusion solution and lasted for 30 min.

ZK 36 374 treatment (0.1 ng/ml) started at the beginning of the control period before cardiac arrest and was continued both in the St Thomas Hospital cardioplegic solution and in the modified Meyler perfusion solution. At the end of the reperfusion period the hearts were weighed and dried at 100°C to constant weight to provide the wet weight : dry weight ratio indicating the extent of oedema formation.

Left ventricular pressure (LVP) was measured by means of a catheter inserted into the left ventricle via the mitral ostium and connected to a Statham pressure transducer. An ECG was measured by means of two

silver electrodes: one attached to the metal inflow cannula and the other to the ventricular apex. Heart rate (HR), PQ-interval and the occurrence of arrhythmias were monitored by this ECG to evaluate the electrophysiologic function of the heart.

The pressure-rate index was calculated as the product of LVP and HR, and was taken as an indicator for myocardial function. Coronary flow was calculated by a microprocessor, which kept the perfusion pressure constant by controlling the peristaltic perfusion pump (LKB, microperpex).

Overflow of adenosine and its catabolites was used as a sensitive indicator for nucleotide breakdown and hence, for ischemic damage (3,7). These purines were assayed with a slightly modified version of the high-performance liquid chromatography assay as described by Harmsen et al. (5). All data are expressed as the mean ± S.E.M. (control group; n=6; ZK 36 374 group; n=5). A comparison between the groups was made by Student's t-test.

During the pre-ischemic control period, none of the measured parameters was significantly altered by ZK 36 374 , e.g. heart rate 298 ± 7 to 314 ± 13 beats/min; coronary flow 9.7 ± 0.8 to 11.0 ± 1.8 ml/min; LVP 25 ± 3 to 22 ± 3 mmHg; pressure-rate index 7.5 ± 0.8 . 10^3 to 7.2 ± 1.1 . 10^3 mmHg/min and total purine overflow 10 ± 1 to 12 ± 2 nmol/min gdwt. Apparently, all hearts were in a similar condition at the start of the ischemic period, i.e. drug treatment did not affect the pressure-rate index and hence the oxygen consumption (4).

After 24 h hypothermic cardiac arrest, coronary flow was decreased in both groups, however in the drug treated hearts this decrease was

significantly less than in the control hearts (Fig. 1). The mechanical performance of both groups was also impaired after hypothermic cardiac arrest. However, the recovery of the pressure-rate index of the treated hearts was significantly higher (51 ± 7%) as compared to the control hearts (17 ± 6%) (Fig. 1). Oedema formation was not significantly altered by drug treatment. (Wet wt. : dry wt. ratio = 5.7 ± 0.1 in treated hearts and 5.5 ± 0.3 in control hearts).

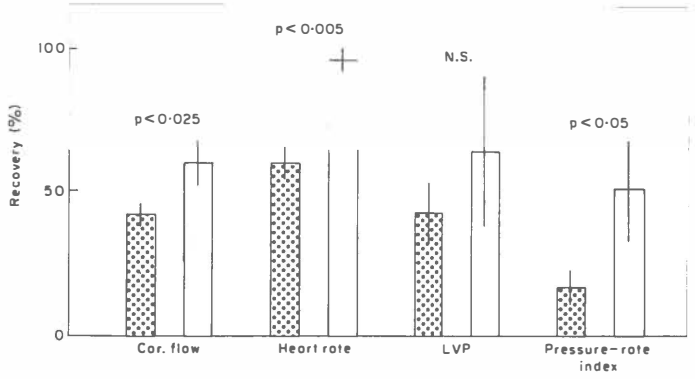

FIGURE 1. The effects of ZK 36 374 on the recovery of coronary flow, heart rate, left ventricular pressure (LVP) and pressure-rate index. The data, stable after 30 min of reperfusion, are expressed as percentage of the pre-ischemic value. (N.S. = not significant.) ▨, control; □, ZK 36 374.

On reperfusion purine overflow was significantly less in the treated hearts (369 ± 36 to 594 ± 14 nmol/min gdwt) (Fig. 2). Reduction of inosine overflow was most pronounced (336 ± 33 to 188 ± 36 nmol/min gdwt) (Fig. 2). This profound decrease in purine overflow was only seen in the first minute of reperfusion. Five minutes later the differences in purine overflow were not significantly anymore. After 30 min reperfusion total purine overflow of control and treated hearts was

90 ± 8 resp. 85 ± 3 nmol/min gdwt. This indicates that ZK 36 374 treatment reduces myocardial adenine nucleotide breakdown and hence ischemic damage during hypothermic cardiac arrest.

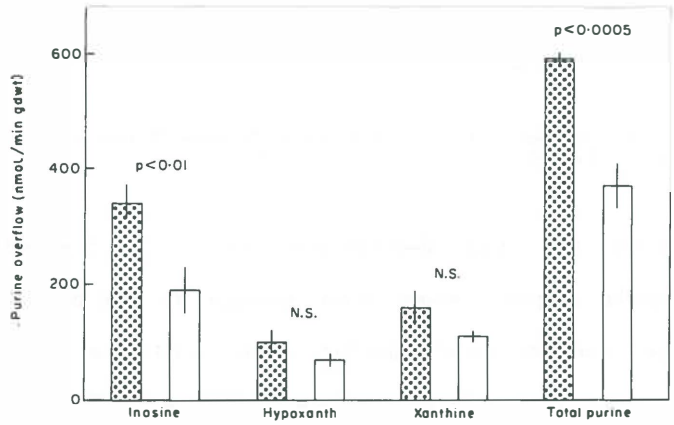

FIGURE 2. The effects of ZK 36 374 on the initial overflow of inosine, hypoxanthine and xanthine. The adenine nucleotide metabolites were assayed in the coronary effluent collected in the first minute of reperfusion and expressed as nmol/min gdwt. (N.S. = not significant.) ▨, control; □, ZK 36 374.

During the reperfusion period severe arrhythmias were observed in the control hearts. These arrhythmias were multifocal and two control hearts deteriorated rapidly into ventricular fibrillation, which lasted for the whole reperfusion period. However, the drug treated hearts showed less arrhythmias and periods of stable ventricular tachycardia occurred in two hearts only. Ventricular fibrillation was not observed in any of the drug treated hearts. Recovery of sinus- and AV-nodal function was complete for drug treated hearts because they were all in sinus rhythm at their initial frequency and without an alteration of the PQ interval (Fig. 3). In contrast, the control hearts had second and higher degree AV-block (Fig. 3) with an average ventricular rate of 60 ± 6% of control (Fig. 1). Therefore, ZK 36 374 improved electrophysiological recovery (function of sinus- and AV-node and less arrhythmias).

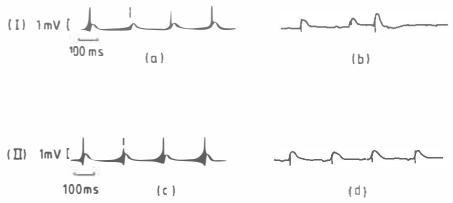

FIGURE 3. Typical ECG recordings of a control (I) and a ZK 36 374 treated (II) heart at the end of the control perfusion period (a and c) and after 30 min of reperfusion (b and d).

In conclusion, our study demonstrates that a 24 h period of hypothermic cardiac arrest causes sever damage to the mechanical, electrophysiologic and metabolic properties of the isolated rat heart. Moreover, it shows that a stable prostacyclin analogue (ZK 36 374) greatly improves myocardial recovery after 24h hypothermic cardiac arrest. Whether this protection is the result of an antiadrenergic and/or cytoprotective activity cannot be concluded from these preliminary results. However, our data indicate that ZK 36 374 acts mainly during the hypothermic arrest period, which supports the idea that it reduces the deleterious ischemia-induced destruction of adrenergic nerve endings (13).

REFERENCES

1. Aiken JW, Gosman RR, Shebushi RJ. Prevention of blockade of partially obstructed coronary arteries with prostacyclin correlates with inhibition of platelet aggregation. Prostaglandins 17, 483-494, 1979.
2. Arahi H, Lefer AM. Role of prostacyclin in the preservation of ischemic myocardial tissue in the perfused cat heart. Circ Res 47, 757-763, 1980.
3. Gilst WH van, Langen CDJ de. Ischemia-reperfusion induced arrhythmias in the isolated rat heart. Pharm Weekbl Sci Ed 4, 160, 1982.
4. Gobel FL, Nordstrom LA, Nelson RR, Jorgenson CR, Wang Y. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 57, 549-556, 1978.
5. Harmsen E, Jong JW de, Serruys PW. Hypoxanthine production by ischemic heart demonstrated by high performance liquid chromatography of blood purine necleosides and oxypurines. Clin Chim Acta 115, 73, 1981.
6. Hearse DJ, Stewart DA, Braimbridge MV. Cellular protection during myocardial ischemia. The development and characterization of a procedure for the induction of reversible ischemic arrest. Circulation 54, 193, 1976.
7. Jong JW de, Verdouw PD, Remme WJ. Myocardial nucleoside and carbohydrate metabolism and hemodynamics during partial occlusion

and reperfusion of pig coronary artery. J Mol Cell Cardiol 9, 297, 1977.

8. Koomen JM, Gilst WH van, Zimmerman ANE, Noordwijk J van. A concentration-dependent biphasic positive inotropic action of ouabain on isolated hearts of rat and guinea-pig. Arch Int Pharmacodyn Ther 255, 2, 1982.
9. Ogletree ML, Lefer AM, Smith JB, Nicolaou KC. Studies on the protective effects of prostacyclin in acute myocardial ischemia. Eur J Pharmacol 56, 95-103, 1979.
10. Ohlendorf R, Perzborn E, Schrör K. Prevention of infarction-induced decrease in circulating platelet count by prostacyclin. Throm Res 19, 447-453, 1980.
11. Schrör K, Darius H, Matzky R, Ohlendorf R. The antiplatelet and cardiovascular actions of a new carbacyclin derivative (ZK 36 374)- equipotent to PGI_2 in vitro. Naunyn-Schmiedebergs Arch Pharmacol 316, 252-256, 1981.
12. Schrör K, Ohlendorf R, Darius H. Beneficial effects of a new carbacyclin derivative, ZK 36 374, in acute myocadial ischemia. J Pharmacol Exp Ther 219, 243-249, 1981.
13. Schrör K, Darius H, Addicks K, Koster R, Smith III EF. PGI_2 prevents ischemia-induced alterations in cardiac catecholamines without influencing nerve stimulation-induced catecholamine release in nonischemic conditions. J Cardiovasc Pharmacol 4, 741-748, 1982.
14. Uchida Y, Murao S. Effects of prostaglandin I_2 on cyclical reductions of coronary blood flow. Jpn Circ J 43, 645-652, 1979.

APPENDIX II

IMPROVED RECOVERY OF CARDIAC FUNCTION AFTER 24 H OF HYPOTHERMIC ARREST IN THE ISOLATED RAT HEART: COMPARISON OF A PROSTACYCLIN ANALOGUE (ZK 36 374) AND A CALCIUM ENTRY BLOCKER (DILTIAZEM).

W.H. van Gilst, P.W. Boonstra, J.A. Terpstra,
Ch.R.H. Wildevuur, C.D.J. de Langen.

Journal of Cardiovascular Pharmacology 7: 520-524, 1985

IMPROVED RECOVERY OF CARDIAC FUNCTION AFTER 24 H OF HYPOTHERMIC ARREST IN THE ISOLATED RAT HEART: COMPARISON OF A PROSTACYCLIN ANALOGUE (ZK 36 374) AND A CALCIUM ENTRY BLOCKER (DILTIAZEM).

SUMMARY

The effects of a stable prostacyclin analogue (ZK 36 374; 4 nM) and a calcium entry blocker (diltiazem; 50 nM) on the recovery of cardiac function after 24 h of hypothermic (10°C) cardiac arrest were studied in the isolated rat heart. Recovery of the pressure-rate index of treated hearts was significantly better (59 ± 10% for diltiazem ($p < 0.05$) and 51 ± 7% for ZK 36 374 ($p < 0.05$)) than in untreated hearts (27 ± 9%). Untreated hearts had second- and higher-degree atrioventricular block, with an average ventricular rate of 60 ± 6% of control. All drug-treated hearts, however, were in sinus rhythm at their initial frequency without a significant alteration in PQ interval. Moreover, the incidence of severe arrhythmias was significantly reduced by ZK 36 374 ($p < 0.02$) and diltiazem ($p < 0.01$). ZK 36 374 reduced total purine overflow upon reperfusion (503 ± 51 vs. 223 ± 22 nmol min^{-1} g dry $weight^{-1}$; $p < 0.0005$). The delayed overflow of adenosine, a proposed marker of reperfusion damage, was not affected by ZK 36 374 treatment. In contrast, diltiazem had no effect on total purine overflow upon reperfusion, but nearly abolished delayed adenosine overflow. It is

concluded from these results that both ZK 36 374 and diltiazem improve myocardial recovery after 24 h of hypothermic cardiac arrest.

INTRODUCTION

Developments in cardiac surgery, such as coronary bypass grafting and ischemic cardiac arrest, have strongly stimulated research in the use of cardioplegic solutions (1-3) and the addition of protective agents to these solutions (4).

The efficacy of these protective agents may not be limited to the ischemic period, since some studies have shown that reperfusion of the ischemic myocardium can lead to a paradoxical extension of the damage resulting from ischemia alone (5-8). The biochemical basis for this finding is not known at present, but is presumably related to a loss in membrane integrity (9) and the inability of the ischemic myocardium to maintain normal calcium homeostasis upon reperfusion (10-12). In this regard, calcium entry blockers are considered to be potentially useful components of cardioplegic solutions, since slow channel blockade may prevent a detrimental accumulation of calcium in jeopardized myocardial cells (13).

By a different mechanism, prostacyclin and a stable prostacyclin analogue, ZK 36 374, were also demonstrated to preserve myocardial cell integrity during ischemia by inhibition of lysosomal enzyme release (14,15). Therefore, these agents may also be a useful addition to cardioplegic solutions (16) and protect myocardial cells by preventing calcium overloading via a different mechanism.

In this study we compared the protective efficacy of a calcium entry blocker, diltiazem, and a stable prostacyclin anlogue, ZK 36 374, in an isolated rat heart model of 24 h of hypothermic cardiac arrest.

Furthermore, we investigated whether their protective effect resulted in less ischemic damage or was mainly due to the prevention of deleterious reperfusion phenomena.

MATERIALS AND METHODS

Male Wistar rats (275-325 g body weight) were anesthetized with diethyl ether and heparinized intravenously. After rapid excision, the hearts were subjected to Langendorff nonrecirculating perfusion and were allowed to beat spontaneously. The perfusate was a modified Meyler bicarbonate buffer (pH 7.4) containing the following (mM): NaCl, 128.2; KCl, 4.7; $CaCl_2$, 1.3; $MgCl_2$, 1.1; NaH_2PO_4, 0.4; $NaHCO_3$, 20.2; and glucose, 10 (16). The solution was gassed at 37°C with 95% O_2 and 5% CO_2. The perfusion pressure was maintained at 60 mmHg.

After a washout and equilibration period of 15 min, control values of coronary flow, left ventricular pressure, and heart rate were recorded for another 15 min. At the end of this control period, cardiac arrest was effected by perfusion of the heart with St. Thomas Hospital solution (4 ml/min; 10°C) (1) for 2 min. Cardiac arrest occurred within 1 min. The St. Thomas Hospital solution contained the following (mM): NaCl, 110.0; KCl, 16.0; $MgCl_2$, 16.0; $CaCl_2$, 1.2; and $NaHCO_3$, 10.0.

Subsequently, the hearts were stored at 10°C for 24 h in 20 ml St. Thomas Hospital solution which was continuously gassed with 95% O_2 and 5% CO_2. Reperfusion of the heart was performed with the modified Meyler

perfusion solution and lasted for 30 min. During the latter period the recovery of cardiac function was monitored.

Since dose-response curves for cardioprotective effects appear to be bell shaped (4), drug concentrations with submaximal effect (15) were chosen. The hearts were divided at random into three groups: untreated group (n=8), ZK 36 374-treated group (4 nM; n=5), and diltiazem-treated group (50 nM; n=7). Drug treatment started at the beginning of the control period and was continued both in the St. Thomas cardioplegic solution and, during the reperfusion period, in the modified Meyler perfusion solution.

At the end of the reperfusion period the hearts were weighed and then dried at 100°C to constant weight.

Left ventricular pressure was measured by means of a catheter inserted into the left ventricle via the mitral valve and connected to a pressure transducer (Statham P23 Db). The cardiac electrogram (ECG) was measured by means of two silver electrodes: one attached to the metal inflow cannula and the other to the ventricular apex. The ECG was monitored continuously and stored on magnetic tape. The ECG was visualized using an ink jet recorder at a paper speed of 100 mm/s (Siemens Oscillomink E). Arrhythmias occurring during reperfusion were categorized as severe or moderate arrhythmias. Ventricular fibrillation and sustained multiform ventricular tachycardia were considered severe arrhythmias. Short runs of uniform tachycardia and ventricular ectopic beats were considered moderate arrhythmias.

The pressure-rate index was calculated as the product of maximal left ventricular pressure and heart rate and was used as an index for myocardial function.

Coronary flow (volume of perfusion fluid per time unit) was measured by means of a microprocessor, which maintained the perfusion pressure at 60 mm Hg with aid of an electronic feedback circuit adjusting the peristaltic perfusion pump (LKB, Microperpex).

The overflow of adenosine and its catabolites (inosine, hypoxanthine, and xanthine) was used as a sensitive indicator of nucleotide breakdown and, hence, of ischemic damage (17). These purines were assayed using a slightly modified version of the high performance liquid chromatography assay as described by Harmsen et al. (18). The detection level for each ATP catabolite was 0.5 μM. Therefore, a minimal purine overflow of $\backsim$0.5 nmol/min/g dry weight could be detected.

Results are expressed as means $\pm$ SEM. Significance values were calculated by Student's t test or Fisher's exact test; differences were considered significant at p values less than 0.05.

RESULTS

Control perfusion period.

The effects of diltiazem and ZK 36 374 on coronary flow, heart rate, pressure-rate index, and total purine overflow at the end of the preischemic control period are depicted in Table 1. At the concentrations used, ZK 36 374 did not affect coronary flow

significantly; diltiazem tended to increase coronary flow, but the change was not significant.

ZK 36 374 did not affect heart rate, but diltiazem decreased the heart rate significantly to 67 ± 8% of control ($p < 0.05$).

The pressure-rate index was decreased by diltiazem to 53 ± 10% of control ($p < 0.05$), but ZK 36 374 had no significant effect.

The purine overflow was near detection level and was not significantly altered by either drug.

TABLE 1. *Effects of ZK 36 374 (4 nM) and diltiazem (50 nM) on isolated rat heart at end of control period*

	Control	ZK 36 374	Diltiazem
Coronary flow (ml/min)	9.9 ± 0.6	11 ± 1.8	10.9 ± 0.3
Heart rate (beats/min)	307 ± 8	314 ± 13	223 ± 28[a]
Pressure–rate index (% of t = 0)	109 ± 6	106 ± 9	53 ± 10[a]
Purine overflow (nmol min^{-1} g dry weight^{-1})	5 ± 2	9 ± 3	10 ± 3

[a] Indicates a significant ($p < 0.05$) change when compared with untreated hearts.

Reperfusion period after 24 h of hypothermic cardiac arrest.

After 24 h of hypothermic cardiac arrest, coronary flow was decreased in all three groups. However, in the ZK 36 374-treated hearts this decrease was significantly less than in the untreated hearts (Fig. 1). The pressure-rate index was also impaired in all three groups; however this impairment was significantly less in the diltiazem- and ZK 36 374-treated groups (59 ± 10 and 51 ± 7%, respectively) when compared with the untreated group (27 ± 9%; Fig. 2).

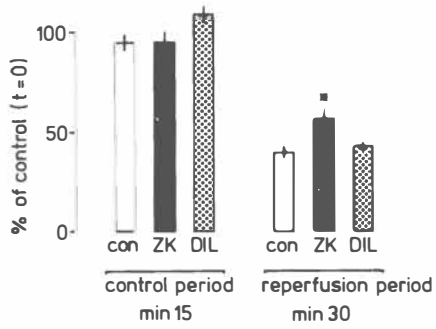

FIG. 1. Effects of ZK 36 374 (ZK; 4 n*M*) and diltiazem (DIL; 50 n*M*) on coronary flow at end of control and reperfusion periods. Measured values are expressed as a percentage of the value at t = 0, and columns represent means ± SEM. Asterisk indicates significant alteration compared with control group ($p < 0.05$).

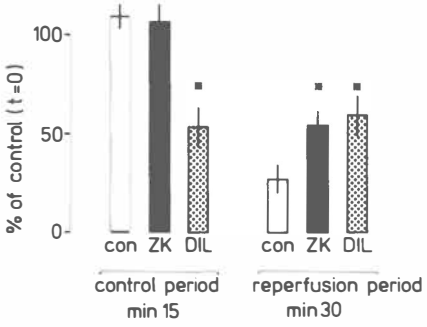

FIG. 2. Effects of ZK 36 374 (ZK; 4 n*M*) and diltiazem (DIL; 50 n*M*) on pressure–rate index at end of control and reperfusion periods. Measured values are expressed as a percentage of the value at t = 0, and the columns represent means ± SEM. Asterisks indicate significant alteration compared with control group ($p < 0.05$).

On reperfusion, total purine overflow was significantly less in the ZK 36 374-treated hearts (223 ± 22 vs. 503 ± 51 nmol min^{-1} g dry $weight^{-1}$; Fig. 3). This profound decrease in total purine overflow was only seen in the 1st min of reperfusion. Five minutes later the

differences in purine overflow were not significant anymore. ZK 36 374 treatment did not affect the adenosine overflow during reperfusion (Fig. 3).

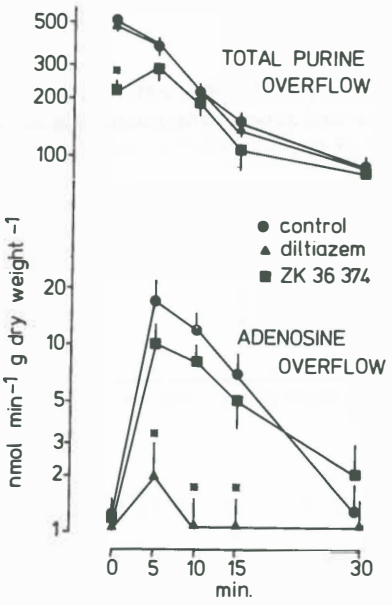

FIG. 3. Time course of overflow of total purines and adenosine alone during reperfusion period for untreated (circle), diltiazem- (triangle), and ZK 36 374-treated (square) hearts. Measured values are calculated as nanomoles per minute, expressed per gram dry weight, and plotted against time on a semilogarithmic scale. Symbols represent means ± SEM. Asterisks indicate significantly altered overflow compared with control group ($p < 0.05$).

In marked contrast, diltiazem reduced the adenosine overflow to hardly detectable levels (Fig. 3). The overflow of inosine, hypoxanthine, and xanthine upon reperfusion was not significantly different from the untreated group. Moreover, the efflux pattern of

these adenosine catabolites was comparable with that of untreated hearts (Fig. 3)

During the reperfusion period severe arrhythmias were observed in the untreated hearts (Table 2). Three untreated hearts deteriorated rapidly into ventricular fibrillation, which lasted for the whole

TABLE 2. *Effects of ZK 36 374 (4 nM) and diltiazem (50 nM) on incidence of moderate and severe arrhythmias during reperfusion period after 24 h of hypothermic cardiac arrest*

		Hearts with:		
	(n)	Moderate arrhythmias	Severe arrhythmias	
Control	(8)	3	5	
ZK 36 374	(5)	5	0	$p < 0.02$[a]
Diltiazem	(7)	7	0	$p < 0.01$[a]

[a] When compared with control group (Fisher's exact test).

reperfusion period. These three hearts were omitted when heart rate and pressure-rate index were calculated (Figs 2 and 4). In contrast, the drug-treated hearts showed only moderate arrhythmias. Ventricular fibrillation was not observed in any of the drug-treated hearts. A total recovery of sinus and atrioventricular (AV) nodal function was observed in the ZK 36 374-treated hearts because they were all in sinus rhythm at their initial frequency (Fig. 4) and without an alteration of the PQ interval.

Diltiazem-treated hearts were also in sinus rhythm at the same frequency at the end of the control period, with a slight increase of the PQ interval (from 49 ± 2 to 54 ± 3 ms; NS). In contrast, the

untreated hearts had second- and higher-degree AV block, with an average ventricular rate of 60 ± 6% of control (Fig. 4).

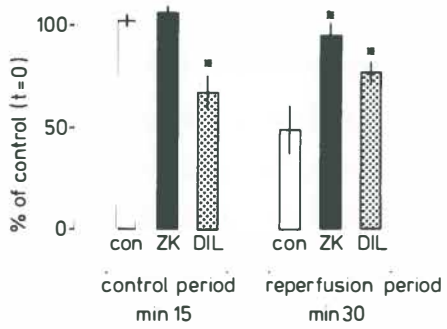

FIG. 4. Effects of ZK 36 374 (ZK; 4 n*M*) and diltiazem (DIL; 50 n*M*) on heart rate at end of control and reperfusion periods. Measured values are expressed as a percentage of the value at t = 0, and columns represent means ± SEM. Asterisks indicate significant alteration compared with control group ($p < 0.05$).

DISCUSSION

This study demonstrates that a 24 h period of hypothermic cardiac arrest causes severe damage to the mechanical and electrophysiologic function of the isolated rat heart. Moreover, it shows that both a stable prostacyclin analogue (ZK 36 374) and a calcium entry blocker (diltiazem) improve mechanical and electrophysiologic recovery after 24 h of hypothermic cardiac arrest.

During the 1st min of reperfusion, a massive overflow of inosine, hypoxanthine, and xanthine was detected in the cardiac effluent of

untreated hearts. No adenosine overflow was observed at this moment. Presumably, the adenosine leaked from the ischemic cells is converted to inosine during cardiac arrest, i.e., the high rate of adenosine deamination (19,20) to inosine is probably responsible for this lack of adenosine overflow. In subsequent samples, a delayed adenosine overflow was observed for untreated hearts. Apparently, a washout of these ATP catabolites accumulated during the cardiac arrest period represents an increased nucleotide breakdown of myocardial cells. The delayed adenosine overflow, however, might indicate additional reperfusion damage in this model.

Interesting differences in the drug effects on purine overflow upon reperfusion were found in this study. Although the kinetics of ATP catabolism during ischemia and reperfusion are not known in sufficient detail, a tentative interpretation is possible. For instance, the prostacyclin analogue, ZK 36 374, is associated with a decreased initial washout of purines. It is proposed that this agent protects myocardial cells during the cardiac arrest period, probably by preserving membrane integrity (14,15). However, this compound seems to lack protective properties during reperfusion. In its presence the delayed overflow of adenosine was not prevented. The elevated ATP catabolism, which might be due to calcium overloading (10-13) of myocardial cells, was not affected.

In marked contrast, diltiazem had no significant effect on total purine overflow during the 1st min of reperfusion and, hence, did not improve the conservation of high-energy phosphate nucleotides during the cardiac arrest period. However, diltiazem was associated with nucleotide

sparing during reperfusion, which was reflected in an abolishment of the delayed adenosine overflow. Whether this effect is caused by an enhanced adenosine reuptake or by a decreased ATP catabolism by preventing the calcium overload of jeopardized myocardial cells cannot be concluded from our experiments.

If this proposed concept of protective mechanisms could be confirmed in further studies, it is to be expected that the sequential use of a prostacyclin-mimetic compound during cardiac arrest and a calcium entry blocker during reperfusion may have a synergistic effect on the preservation of myocardial cell function during cardioplegia.

REFERENCES

1. Hearse DJ, Stewart DA, Braimbridge MV. Cellular protection during myocardial ischemia. The development and characterization of a procedure for the induction of reversible ischemic arrest. Circulation 54: 193-202, 1976.
2. Bretschneider HJ, Hübner G, Knoll D, Lohr B, Nordbeck H, Spieckerman PG. Myocardial resistance and tolerance to ischemia. Physiological and biochemical basis. J Cardiovasc Surg (Torino) 16: 241-260, 1975.
3. Fisk RL, Gelfand ET, Callaghan JC. Hypothermic coronary perfusion for intra-operative cardioplegia. Ann Thorac Surg 23: 56-61, 1977.
4. Hearse DJ, O'Brien K, Braimbridge MV. Protection of the myocardium during ischemic arrest. Dose-response curves for procaine and lignocaine in cardioplegic solutions. J Thorac Cardiovasc Surg 81: 873-879, 1981.
5. Bulkley BH, Hutchins GM. Myocardial consequences of coronary artery bypass graft surgery: the paradox of necrosis in areas of revascularization. Circulation 56: 906-913, 1977.
6. Buckberg GD. A proposed 'solution' to the cardioplegic controversy. J Thorac Cardiovasc Surg 77: 803-815, 1979.
7. Reimer KA, Hill ML, Jennings RB. Prolonged depletion of ATP and of the adenine nucleotides following reversible myocardial ischemic injury in dogs. J Mol Cell Cardiol 13: 229-239, 1981.
8. Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 66: 1146-1149, 1982.

9. Sobel BE, Corr PB, Robison AK, Goldstein RA, Witkowski FX, Klein MS, Accumulation of lysophosphoglycerides with arrhythmogenic properties in ischemic myocardium. J Clin Invest 62, 546-553, 1978.

10. Hearse DJ. Editorial: reperfusion of the ischemic myocardium. J Mol Cell Cardiol 9: 605-616, 1977.

11. Nayler W.G. The role of calcium in the ischemic myocardium. Am J Phathol 102: 262-270, 1981.

12. Murphy ML, Peng CF, Kane JJ, Straub KD. Ventricular performance and biochemical alteration of regional ischemic myocardium after reperfusion in the pig. Am J Cardiol 50: 821-828, 1982.

13. Henry PD, Schuchleib R, Davis J, Weiss ES, Sobel BE. Myocardial contracture and accumulation of mitochondrial calcium in ischemic rabbit heart. Am J Physiol 233: H677-684, 1977.

14. Ogletree ML, Lefer AM, Smith JB, Nicolaou KC. Studies on the protective effect of prostacyclin in acute myocardial ischemia. Eur J Pharmacol 56: 95-103, 1979.

15. Schrör K, Ohlendorf R, Darius H. Beneficial effects of a new carbacyclin derivative, ZK 36 374, in acute myocardial ischemia. J Pharmacol Exp Ther 219: 243-249, 1981.

16. van Gilst WH, Boonstra PW, Terpstra JA, Wildevuur ChRM, de Langen CDJ. Improved functional recovery of the isolated rat heart after 24 hours of hypothermic arrest with a stable prostacyclin analogue (ZK 36 374). J Mol Cell Cardiol 15: 789-792, 1983.

17. de Jong JW, Verdouw PD, Remme WJ. Myocardial nucleoside and carbohydrate metabolism and hemodynamics during partial occlusion and reperfusion of pig coronary artery. J Mol Cell Cardiol 9: 297, 1977.

18. Harmsen E, de Jong JW, Serruys PW. Hypoxanthine production by ischemic heart demonstrated by high performance liquid chromatography of blood purine nucleosides and oxypurines. Clin Chim Acta 115: 73, 1981.

19. Berne RM, Rubio R. Adenine nucleotide metabolism in the heart. Cir Res 34 and 35, (suppl 3) 109-120, 1974.

20. de Jong JW. Phosphorylation and deamination of adenosine by the isolated perfused rat heart. Biochim Biophys Acta 286: 252-259, 1972.

APPENDIX III

CAPTOPRIL REDUCES PURINE LOSS AND REPERFUSION ARRHYTHMIAS IN THE RAT HEART AFTER CORONARY ARTERY OCCLUSION

W.H. van Gilst, P.A. de Graeff, J.H. Kingma,
H. Wesseling and C.D.J. de Langen.

European Journal of Pharmacology, 100: 113-117, 1984

CAPTOPRIL REDUCES PURINE LOSS AND REPERFUSION ARRHYTHMIAS IN THE RAT HEART AFTER CORONARY ARTERY OCCLUSION

SUMMARY

Captopril was perfused through isolated rat hearts; its effects after local ischemia and reperfusion were assessed. Upon reperfusion all untreated (10 out of 10) but only 4 (out of 10) captopril-treated (80 μg/ml) hearts fibrillated ($p < 0.02$). Purine overflow increased upon reperfusion but was reduced by captopril (597 ± 62 and 333 ± 41 nmol/min gdwt respectively; $p < 0.05$). The pressure-rate index and the apex displacement were severely impaired after 30 min of reperfusion (32 ± 16 and 10 ± 5% respectively of initial values) but captopril reduced the injury of mechanical function (60 ± 8; $p < 0.05$ and 61 ± 11; $p < 0.05$ respectively). These results show that captopril reduces ventricular fibrillation and the loss of high energy phosphate nucleotides and thereby partly maintains mechanical function impaired by ischemia and reperfusion.

INTRODUCTION

Relaxation of coronary arterial spasm, leading to reperfusion-induced ventricular fibrillation has been suggested as a major cause of sudden cardiac death in man (Axelrod et al., 1975; Hearse, 1977).

The mechanisms of reperfusion arrhythmais are not fully understood. It has been suggested that a massive local release of catecholamines plays an important role in the generation of reperfusion arrhythmias (Lown and Verrier, 1976) and that alpha-adrenergic effects are crucial in the chain of events leading to ventricular fibrillation (Corr and Crafford, 1981). Furthermore, it is known that the synthesis and release of catecholamines are increased by angiotensin II (Starke, 1971).

Captopril not only inhibits converting enzyme but also may interfere with the neurogenic noradrenaline transmission in a manner that is independent of angiotensin II (Clough et al., 1982; Saruta et al., 1982). Therefore, the purpose of the present study was to evaluate the effects of captopril on the incidence of arrhythmias during reperfusion after local ischemia in the isolated rat heart.

MATERIALS AND METHODS

Reperfusion model

Male Wistar rats (275-325 g), fed ad libitum, were anesthetized with ether and given 500 I.U. of heparin intravenously. The hearts were

rapidly excised and arrested in ice cold 0.9% NaCl. Retrograde perfusion of the aorta as described by Langendorff was immediately started using a modified Tyrode solution containing 10 mM glucose and equilibrated with a mixture of 95% O_2 + 5% CO_2. The perfusion pressure was maintained at 60 mmHg with an electronic feedback circuit to the perfusion pump. The temperature was kept between 36.5 and 37.5°C. The hearts beated spontaneously.

Acute regional myocardial ischemia was produced as described by Lubbe et al. (1978). The descending branch of the left coronary artery was ligated with 6-0 silk 2 mm below the aortic root using a 3/8 circle taper point needle (Ethicon). Reperfusion of the ischemic tissue was achieved by releasing the ligation. This technique of reversible ligation of the left coronary artery has proven to be a useful model to provoke ventricular arrhythmias (Lubbe et al., 1978)

Protocol

The hearts were allowed to equilibrate with the perfusion fluid for 15 min. After this equilibration period and a control perfusion of 15 min, local ischemia was provoked for the next 15 min. After reperfusion of the ischemic zone for 30 min the experiments were discontinued.

The rat hearts were divided at random into two groups of 10 each. In one group, captopril was added to the perfusion fluid at the start of the control period in a concentration of 80 μg/ml. Captopril was present throughout the whole experiment including the period of reperfusion.

Measurement of mechanical and electrophysiological parameters

Apex displacement and the pressure-rate product were used as indices of contractility. Apex displacement was measured with a displacement transducer (Hottinger Baldwin, W10) fitted with a steel tampon hooked to the apex of the ventricle. Left ventricular pressure (LVP) was measured by means of a catheter inserted into the left ventricle via the mitral valve and connected to a pressure transducer (Statham P23 Db). The pressure-rate index was calculated as the product of LVP and heart rate. Apex displacement and pressure-rate data during the experiment were calculated as a percentage of the values at the end of the equilibration period.

A bipolar cardiac electrogram was obtained by means of two silver electrodes: one attached to the metal inflow cannula and the other to the ventricular apex. Heart rate, PQ interval and the occurrence of arrhythmias were monitored by continuous registration of the electrogram in order to evaluate the electrophysiologic function of the heart. The recordings were stored on magnetic tape and heart rhythm was visualized using an ink jet recorder at a paper speed of 100 mm/s (Siemens Oscillomink E).

Coronary flow (volume of perfusion fluid per time unit) was measured by a microprocessor, which maintained the perfusion pressure by adjusting the peristaltic perfusion pump (LKB, microperpex).

Assessment of cellular damage

Overflow of adenosine and its catabolites (inosine, hypoxanthine and xanthine) was used as a sensitive indicator of nucleotide breakdown (De Jong et al., 1982). One-minute fractions of the perfusate were collected in ice cold tubes during the whole experiment. The purine nucleosides and oxypurines were determined by a high-performance liquid chromatography assay. At the end of the experiment, the hearts were dried to constant weight and the purine overflow was expressed as nmol/min g dry weight (gdwt).

Reagents

All chemicals were analytical grade. Captopril was a gift from Squibb. Fresh solutions were prepared daily.

Statistical analysis

The data are expressed as the means $\pm$ SEM. Probability values were calculated by Student's t-test or Fisher's exact probability test and P values less than 0.05 were considered to be significant.

RESULTS

Control period

At the end of the control period none of the measured parameters was significantly altered by captopril (table 1). Apparently all hearts were in a similar condition at the start of the ischemic period.

TABLE 1

Effect of 80 μg/ml captopril on the isolated rat heart during control perfusion, ischemia and reperfusion. Effect of 80 μg/ml captopril on several cardiac parameters is shown at the end of the control period (min 15) at the end of the ischemic period (min 30) after 30 min reperfusion (min 60).

	Control. perfusion min 15		Ischemia min 30		Reperfusion min 60	
	Contr.	Captopril	Contr.	Captopril	Contr.	Captopril
Cor. flow (ml/min)	10.3 ± 0.5	9.4 ± 0.7	5.5 ± 0.4	5.6 ± 0.7	9.4 ± 0.5	8.3 ± 0.8
Heart rate (sinus beats/min)	291 ± 11	319 ± 13	273 ± 13	293 ± 13	300 ± 20	309 ± 18
Apex displacement (% of t = 0)	101 ± 6	103 ± 7	23 ± 3	31 ± 4 [a]	10 ± 5	61 ± 11 [a]
Pressure-rate index (% of t = 0)	98 ± 7	103 ± 6	52 ± 9	81 ± 19 [a]	32 ± 16	60 ± 8 [a]
Purine overflow (nmol/min-gdwt)	14 ± 2	22 ± 7	82 ± 6	70 ± 11	62 ± 10	28 ± 9 [a]
PQ interval (ms)	48 ± 1	48 ± 2	49 ± 2	47 ± 2	51 ± 2	48 ± 2

[a] Indicates a significant ($P < 0.05$) change as compared to control values.

Ischemia period

Ligation of the left coronary artery resulted in a comparable flow reduction to 5.5 ± 0.4 ml/min for control hearts and 5.6 ± 0.7 ml/min for captopril-treated hearts. The mechanical performance in both groups was impaired after 15 min of regional ischemia. However, apex displacement and pressure-rate index of the captopril-treated hearts were significantly higher than those of the control hearts. Both groups showed a slight and not significant decrease in heart rate without change of the PQ interval (table 1). Purine overflow increased during coronary ligation in both groups and after 15 min of ischemia amounted

to 82 ± 6 nmol/min gdwt for control hearts and 70 ± 11 nmol/min gdwt for captopril-treated hearts (n.s.; table 1).

Reperfusion period

Release of the coronary ligation resulted for both groups in an immediate and total recovery of the coronary flow (10.6 ± 0.7 ml/min for control hearts and 10.3 ± 0.8 ml/min for treated hearts one min after reperfusion).

At the onset of reperfusion, all control hearts (n=10) showed ventricular fibrillation which lasted for 72 ± 11% of the 30 min reperfusion period. In contrast, captopril treatment significantly reduced the incidence of ventricular fibrillation after restoration of flow. Ventricular fibrillation occurred in only four captopril-treated hearts (n=10; $p < 0.02$). Moreover, the duration of ventricular fibrillation in these hearts was significantly shorter (35 ± 9% of the total reperfusion period; $p < 0.05$).

The effects on mechanical function after 30 min of reperfusion differed between the two groups. In the control group apex displacement and pressure-rate index deteriorated further when compared with the preceding ischemia period. In the captopril group, however, apex displacement recovered from 31 ± 4 to 61 ± 11 and the pressure-rate index decreased insignificantly.

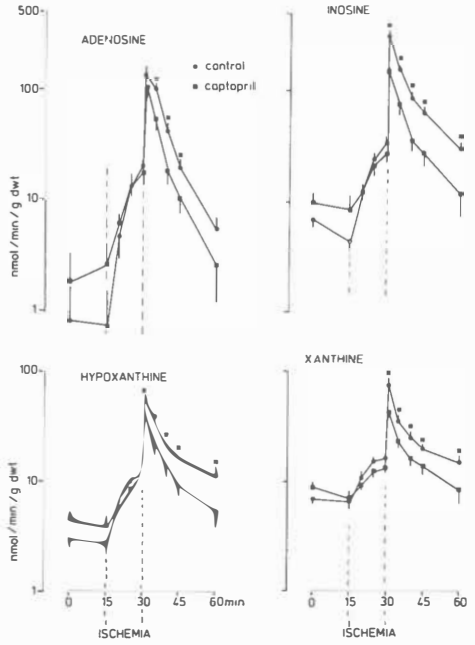

Fig. 1. The overflow of purine nucleosides (upper two panels) and oxypurines (lower two panels) during the experiment for the control (●———●) resp. captopril group (■———■) are plotted against time (min, *abscissa*) on a semi-logarithmic scale. The ischemia period is indicated by a hatched bar on the abscissa. The measured values are calculated as nmol per min and expressed per gram dry weight (gdwt). Symbols represent means ± S.E.M. (n = 10). * Significantly higher overflow when compared with the captopril group ($P < 0.05$).

In the first min of reperfusion total purine overflow was significantly less in the captopril treated hearts (333 ± 41 vs 597 ± 62 nmol/min gdwt) (see overflow patterns of purines and oxypurines in fig. 1). Interestingly a significant difference in purine and oxypurine overflow was observed during the whole reperfusion period but not during the preceding ischemia period. Apparently the multiphasic overflow patterns are composed of an immediate wash-out of purines accumulated

during the ischemia period and a secondary component caused by reperfusion damage.

DISCUSSION

In this study we showed that captopril markedly reduces the incidence and duration of ventricular fibrillation upon reperfusion after 15 min of local ischemia produced by coronary artery ligation in the isolated rat heart. Captopril appears to protect myocardial cells damaged by reperfusion as is obvious from the reduced purine overflow. This purine overflow has proven to be a reliable parameter for the degree of cellular damage (De Jong et al., 1982). Captopril apparently protects the mechanical function during reperfusion by this reduction of cellular damage.

The interesting difference between ischemia- and reperfusion-related events has been noted by other authors (see discussion by Corr and Crafford, 1981). A striking aspect here is the role of the excessive local noradrenaline release presumably triggered by depolarizing amounts of potassium ions leaked from damaged cells and accumulated in the ischemic region. Even in the isolated (and hence acutely denervated) heart residual stored catecholamines may have important deleterious effects whereas chronic neural ablation reduces the incidence of VF. Excessive amounts of noradrenaline may cause an overloading of myocardial cells with calcium ions as the final common pathway of many events leading to VF (Nayler, 1980).

It is tempting to suggest that captopril possesses angiotensin II-independent anti-adrenergic properties (Clough et al., 1982; Saruta et al., 1982) that cause its anti-fibrillatory effect in the isolated heart. Captopril might also act by reducing the angiotensin II concentrations modulating the noradrenergic transmission (Starke, 1971) in vivo. This seems unlikely here since the presence of angiotensin I in the isolated heart has not yet been demonstrated.

In conclusion, our results show marked protective effects of captopril in an isolated heart model of ischemia-reperfusion-induced malignant ventricular arrhythmias at concentrations of captopril well above therapeutic plasma levels. If these results could be extrapolated to the setting of ischemic heart disease in man captopril might be of therapeutic benefit in the early stages of myocardial infarction.

REFERENCES

Axelrod PJ, Verrier RL, Lown B. Vulnerability to ventricular fibrillation during acute coronary arterial occlusion and release. Am J Cardiol 36, 776, 1975.

Clough DP, Collis MG, Conway J, Hatton R, Keddie JR. Interaction of angiotensin-converting enzyme inhibitors with the function of the sympathetic nervous system. Am J Cardiol 49, 1410, 1982.

Corr PB, Crafford WA Jr. Enhanced alpha-adrenergic responsiveness in ischemic myocardium: Role of alpha-adrenergic blockade. Am Heart J 102, 605, 1981.

De Jong JW, Harmsen E, De Tombe PP, Keyzer E. Nifedipine reduces adenine nucleotide breakdown in ischemic rat heart. Eur J Pharmacol 81, 89, 1982.

Hearse DJ. Editorial. Reperfusion of the ischemic myocardium. J Mol Cell Cardiol 9, 605, 1977.

Lown B, Verrier RL. Neural activity and ventricular fibrillation. N Eng J Med 294, 1165, 1976.

Lubbe WF, Daries PS, Opie LH. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of anti-fibrillatory action of antiarrhythmic agents. Cardiovasc Res 12, 212, 1978.

Nayler WG. Calcium antagonists. Eur Heart J. 1, 225, 1980.

Saruta T, Suzuki H, Okundo T, Kondo K. Effects of angiotensin-converting enzyme inhibitors on the vascular response to norepinephrine. Am J Cardiol 49, 1535, 1982.

Starke K. Action of angiotensin on uptake, release and metabolism of 14C-noradrenaline by isolated rabbit hearts. Eur J Pharmacol 14, 112, 1971.

APPENDIX IV

REDUCTION OF REPERFUSION ARRHYTHMIAS IN THE ISCHEMIC ISOLATED RAT HEART BY ANGIOTENSIN CONVERTING ENZYME INHIBITORS. A COMPARISON OF CAPTOPRIL, ENALAPRIL AND HOE 498

W.H. van Gilst, P.A. de Graeff, H. Wesseling
and C.D.J. de Langen.

Accepted for publication in the
Journal of Cardiovascular Pharmacology

REDUCTION OF REPERFUSION ARRHYTHMIAS IN THE ISCHEMIC ISOLATED RAT HEART BY ANGIOTENSIN CONVERTING ENZYME INHIBITORS. A COMPARISON OF CAPTOPRIL, ENALAPRIL AND HOE 498

SUMMARY

The effects of the angiotensin converting enzyme inhibitors, captopril, enalapril, HOE 498 and its prodrug, on reperfusion arrhythmias after 15 min of coronary ligation were investigated in the isolated rat heart. Drug concentrations were equipotent in their effect on angiotensin I pressor response. Furthermore, the effect of indomethacin on ACE inhibition with captopril was studied. Upon reperfusion ventricular fibrillation occurred in all untreated, all prodrug HOE 498 treated (15 microg/ml) and in 4 out of 6 of the enalapril treated (8 microg/ml) hearts. In contrast, only in 2 out of 6 ($p < 0.002$) of the HOE 498 treated (15 microg/ml) and none ($p < 0.001$) of the captopril treated (80 microg/ml) hearts ventricular fibrillation occurred. A massive purine overflow was observed in untreated hearts upon reperfusion. This overflow was significantly reduced by captopril and HOE 498, whereas enalapril and prodrug HOE 498 had no significant effect. Concomitantly, the pressure-rate index was severely impaired after 30 min of reperfusion in the untreated, enalapril and prodrug HOE 498 groups (33 ± 9, 52 ± 11 and 48 ± 12 of initial values respectively) but captopril and HOE 498 significantly reduced the impairment of

mechanical function (124 ± 9% and 98 ± 9% respectively). In contrast to enalapril and prodrug HOE 498, captopril and HOE 498 markedly reduced noradrenaline overflow during the first min of reperfusion. No angiotensin II was detectable in the coronary effluent of untreated hearts. All the beneficial effects of captopril were abolished by simultaneous administration of indomethacin (1 microM) and no significant differences were observed for these hearts when compared to untreated hearts. It is concluded that captopril and HOE 498 protect against reperfusion arrhythmias. Apparently this effect is independent of inhibition of the formation of angiotensin II and associated with an abolishment of noradrenaline overflow upon reperfusion. Stimulation of prostacyclin synthesis appears to play an important role.

INTRODUCTION

Elucidation of reperfusion phenomena has taken on new significance because of recent clinical evidence that sudden restoration of coronary blood flow can result in serious structural and functional derangements leading to ventricular fibrillation (1, 2, 3, 4).

The role of adrenergic, biochemical and metabolic influences on reperfusion induced ventricular arrhythmias has not been clearly defined. However, it is evident that reperfusion arrhythmias are quite distinct from those associated with ischemia (5). There appears to be a relationship between the onset of irreversible injury during the ischemic period and the occurrence of ventricular reperfusion arrhythmias (6).

Recently we have reported beneficial effects of captopril in an isolated heart model of malignant reperfusion arrhythmias (7). It was proposed that this protective effect was caused by an angiotensin II independent mechanism. The demonstration that captopril may interfere directly with the neurogenic noradrenaline transmission (8) supports this hypothesis.

The present study was undertaken to investigate whether this protective effect of captopril is also present with other non-sulfhydryl angiotensin converting enzyme inhibitors, such as enalapril and HOE 498. These compounds are prodrugs which must be de-esterified first before they become pharmacologically active. In this study the active diacid forms of enalapril and HOE 498 were used. In order to assess the role of angiotensin converting enzyme inhibiting activity the prodrug of HOE 498

was also studied and angiotensin II levels were measured. Furthermore, noradrenaline release and its modulation by angiotensin converting enzyme inhibitors were studied.

Recently a direct stimulatory action of captopril on prostacyclin synthesis has been shown (9). In order to investigate whether this mechanism plays a role in the earlier observed protective effects of captopril (7) cyclo-oxygenase inhibition was used to test this hypothesis.

MATERIALS AND METHODS

Reperfusion model

Male Wistar rats (275-325 g), fed ad libitum, were anesthetized with ether and given 500 I.U. of heparin intravenously. The hearts were rapidly excised and arrested in icy-cold 0.9% NaCl. Retrograde perfusion of the aorta as described by Langendorff was immediately started with a modified Tyrode solution (10), containing 10 mM glucose and equilibrated with 95% O_2 + 5% CO_2. This perfusion buffer was filtered through 1.2 microm pore size filters before reaching the heart. The perfusion pressure was maintained at 60 mmHg. The temperature was continuously measured in the aorta-cannula tip and kept between 36.5 and 37.5°C. The hearts beated spontaneously.

Acute regional myocardial ischemia was produced as described by Kannengieser et al. (11). The left main coronary artery was ligated with 6-0 silk 2 mm below the aortic root using a 3/8 circle taper point needle (Ethicon). Reperfusion of the ischemic tissue was achieved by releasing the ligation. This technique of reversible ligation of the left coronary artery has proven to be a useful model to provoke ventricular arrhythmias (12, 13).

Protocol

The hearts were allowed to equilibrate with the perfusion fluid for 15 min. After this equilibration period and a control perfusion of 15 min, local ischemia was induced and maintained for the next 15 min. After reperfusion of the ischemic zone for 30 min the experiments were terminated.

The rat hearts were divided at random into groups of 6 each. Drugs were added at the start of the control period and treatment was continued during the whole experiment, including the period of reperfusion.

Drug concentrations in study A were: captopril 80 microg/ml, enalapril 8 microg/ml, HOE 498 15 microg/ml and prodrug HOE 498 15 microg/ml.

Drug concentrations in study B were: captopril 80 microg/ml, indomethacin 1 microM and one group received both captopril and indomethacin in the above mentioned concentrations.

Both in study A and B, one group served as control.

Measurement of mechanical and electrophysiological parameters

The pressure-rate product was used as an index of contractility (14, 15) and hence of oxygen consumption (16, 17). Left ventricular end systolic pressure (LVESP) was measured by means of a catheter inserted into the left ventricle via the mitral valve and connected to a pressure transducer (Statham P23 Db). The pressure-rate index was calculated as the product of maximal LVESP and heart rate. Pressure-rate data during the experiment were calculated as a percentage of the values at the end of the equilibration period.

A bipolar cardiac electrogram was obtained by means of two electrodes: one attached to the metal inflow cannula and the other to the ventricular apex outside the ischemic zone. Heart rate, PQ interval and the occurrence of arrhythmias were monitored by continuous registration of the cardiac electrogram in order to evaluate the electrophysiologic function of the heart. ECG was visualised using an ink jet recorder at a paper speed of 100 mm/s (Siemens Oscillomink E).

Coronary flow (volume of perfusion fluid per time unit) was measured by a microprocessor, which controlled the perfusion pressure by adjusting the peristaltic perfusion pump (LKB, microperpex).

Assay of ATP catabolites, catecholamines and angiotensin II

Overflow of adenosine and its catabolites (inosine, hypoxanthine and xanthine) was used as an indicator of nucleotide breakdown. This has proven to be a reliable parameter for the degree of ischemia-induced

cellular damage (13, 18). One-minute fractions of the perfusate were collected in icy-cold tubes during the whole experiment. The purine nucleosides and oxypurines were determined by a slightly modified version of the high-performance liquid chromatography assay as described by Harmsen et al. (19). Analysis of catecholamines was based on liquid chromatography and electrochemical detection as described by Westerink (20). Angiotensin II levels were measured in control hearts with a commercially available RIA kit (IRE, Fleurus, Belgium). At the end of the experiment, the hearts were dried to constant weight and the purine overflow was expressed as nmol/min g dry weight (g dwt).

Reagents

All chemicals were analytical grade. Captopril was a gift from Squibb, HOE 498 and its prodrug were a gift from Hoechst and enalapril a gift from MSD. Fresh solutions were prepared daily.

Statistical analysis

The data are expressed as the means ± SEM. Group differences were tested with Student's t-test or Mann-Whitney's U-test; p values less than 0.05 double-sided were considered to be significant.

RESULTS

Control perfusion period

At the end of the control period none of the measured parameters was significantly altered by any treatment when compared to the control group . In this period no noradrenaline and angiotensin II were detectable in the coronary effluent of both untreated and treated groups. Apparently all hearts were in a similar condition at the start of the ischemic period.

Ischemic period

Ligation of the left coronary artery resulted in comparable flow reduction for the entire heart to 59 ± 4% of control value for all hearts. After 15 min of regional ischemia the pressure-rate index of all groups was impaired. However, the pressure-rate index of the captopril-treated hearts was significantly less impaired than the pressure-rate index of the control hearts (Table I). All groups showed a slight but insignificant decrease in heart rate without a change of the PQ interval during coronary ligation. Purine overflow increased during coronary ligation in all groups. However, this increase of purine overflow was significantly less for the captopril group as compared to the untreated group. During the ischemic period no overflow of noradrenaline and angiotensin II was detectable in the coronary effluent in any of the investigated groups.

TABLE 1

	I	R
Study A: Effect of captopril, enalapril HOE 498, and its prodrug on the pressure–rate index (% of t = 0) at the end of the ischemic period (I) and at the end of the reperfusion period (R).		
Untreated	44 ± 8	33 ± 9
Captopril	74 ± 11[a]	124 ± 9[a]
Enalapril	30 ± 14	52 ± 11
HOE 498	49 ± 9	98 ± 9[a]
Prodrug HOE 498	45 ± 5	48 ± 12
Study B: Effect of captopril, indomethacin, and captopril plus indomethacin on the pressure–rate index at the end of I and R.		
Untreated	44 ± 6	37 ± 9
Captopril	69 ± 3[a]	107 ± 8[a]
Indomethacin	39 ± 12	42 ± 6
Captopril plus indomethacin	37 ± 8	52 ± 10

[a] A significant change ($p < 0.05$) as compared to the untreated group.

All groups showed a slight increase in ventricular premature depolarizations after 10 min ischemia. However, the incidence of these rhythm disturbances was low and not significantly different between the groups.

Reperfusion period

Release of the coronary ligation resulted in an immediate and total recovery of the coronary flow in all groups. At the onset of reperfusion in study A, all untreated and prodrug-HOE 498 treated hearts showed ventricular fibrillation during 21 ± 4 and 17 ± 2 min respectively. Four of the six enalapril treated hearts fibrillated upon reperfusion during 23 ± 4 min, which is not significantly different from the untreated

group. In contrast, captopril and HOE 498 treatment significantly reduced the incidence and duration of ventricular fibrillation after restoration of flow. Ventricular fibrillation occurred in none of the captopril-treated hearts and in only two HOE 498 treated hearts for a period of 8 ± 2 min (p values < 0.001 resp. < 0.002 compared to controls) (Fig. 1).

At the onset of reperfusion in study B results were obtained for the untreated and captopril group similar to those of study A. The incidence of ventricular fibrillation in the untreated group was again 100% with a duration of 7.8 ± 2.2 min, whereas captopril totally prevented ventricular fibrillation completely. The indomethacine-treated hearts showed invariably ventricular fibrillation upon reperfusion, during 4.2 ± 2.2 min, which is not significantly different from control values. Addition of indomethacin to the captopril treated hearts caused

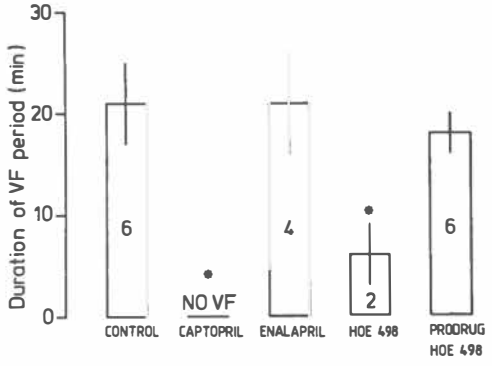

FIG. 1. The incidence and duration (min) of ventricular fibrillation (VF) on reperfusion for treated and untreated hearts are indicated. The values in the vertical bars represent the total number of hearts that fibrillated upon reperfusion in each group (n = 6). *Significant shorter duration of VF when compared with the control group ($p < 0.05$).

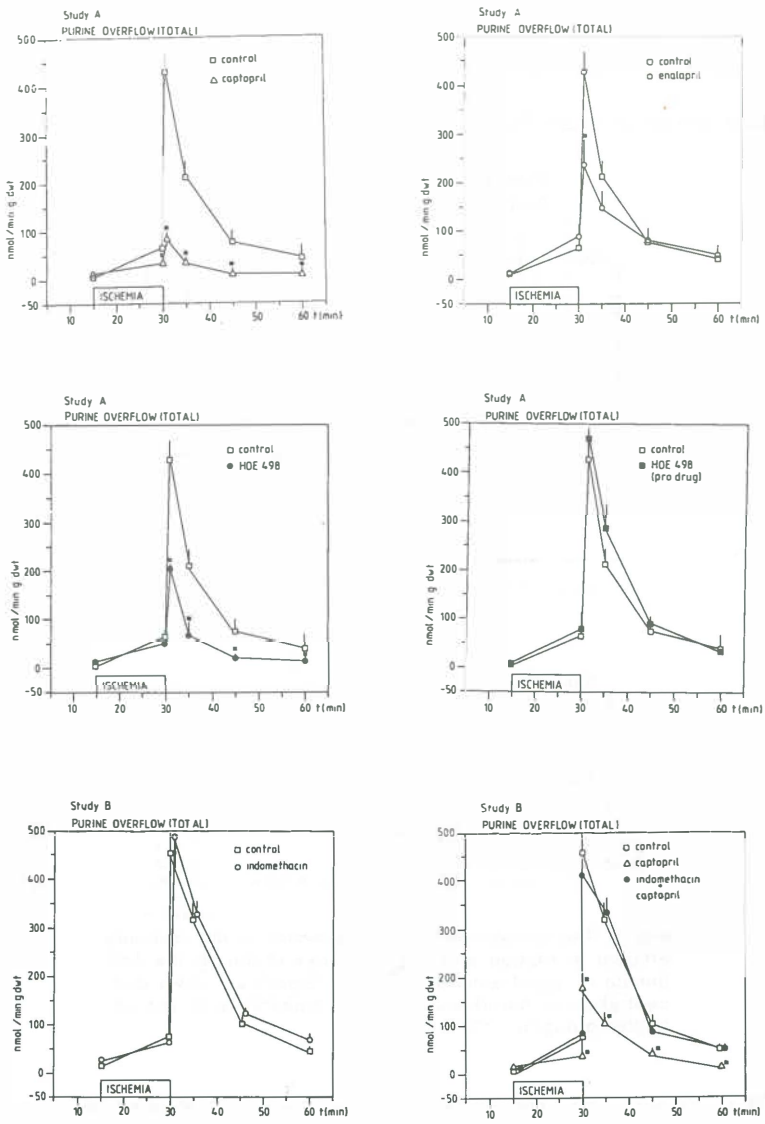

FIG. 2. The total overflow of purine nucleosides and oxypurines during the experiment for treated and untreated hearts are plotted against time (minutes, abscissa). The ischemia period is indicated by a hatched bar on the abscissa. The measured values are calculated as nanomoles per minute and are expressed per gram dry weight (g dwt). Symbols represent means ± SEM ($n = 6$). *Significant lower overflow when compared with the untreated group ($p < 0.05$).

the effects of captopril on reperfusion arrhythmias to disappear. All these hearts showed ventricular fibrillation upon reperfusion for a period of 3.3 ± 1.1 min which is not significantly different from the untreated group in study B.

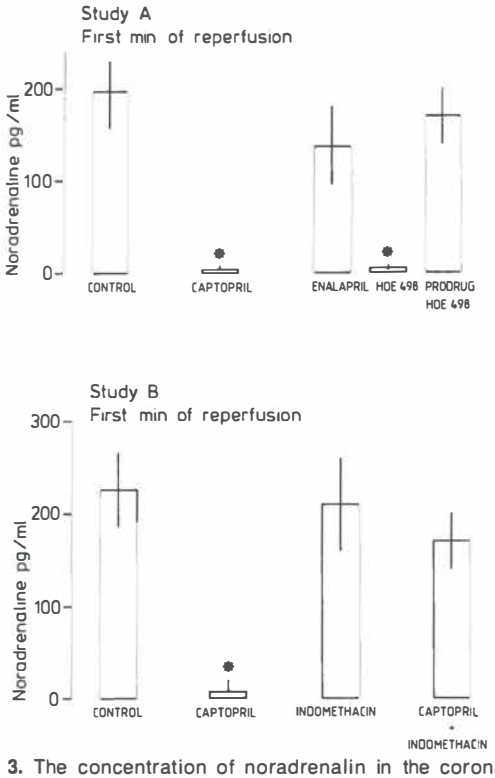

FIG. 3. The concentration of noradrenalin in the coronary effluent of treated and untreated hearts during the first minute of reperfusion is indicated. *Significant lower concentration of noradrenalin when compared with the untreated group ($p < 0.05$).

The effects on the pressure-rate index also showed marked group differences after 30 min of reperfusion. In the untreated, enalapril and prodrug HOE 498 group no significant recovery of the pressure-rate index

was observed when compared to the preceding ischemic period (Table I). In contrast, after 30 min of reperfusion the pressure-rate index in the captopril and HOE 498 treated group had recovered to values that did not differ significantly from the initial values ($p < 0.001$ for both groups when compared to the untreated group). This effect of captopril was also abolished by simultaneous addition of indomethacine (Table I, study B).

Upon reperfusion a massive overflow of purines was detected in all groups (Fig. 2). This purine overflow was significantly reduced by captopril and HOE 498 treatment during the whole reperfusion period. Enalapril treatment resulted in a significant reduction of purine overflow during the first minute of reperfusion only. Prodrug HOE 498 had no significant effect on purine overflow when compared to the untreated group (Fig. 2, study A).

Upon reperfusion a sharp rise in noradrenaline concentration in the coronary effluent was detected in the untreated group (Fig. 3). A similar release of noradrenaline was seen in the enalapril and prodrug HOE 498 group. In contrast, noradrenaline concentrations in the coronary effluent of the captopril and HOE 498 group were below detection level (5 pg/ml) during the first min of reperfusion (Fig. 3, study A).

Indomethacine abolished these effects of captopril, both on purine overflow (Fig. 2, study B) and on noradrenaline overflow (Fig. 3, study B). During reperfusion, the angiotensin II levels remained below detection level (35 pg/ml) in untreated hearts.

DISCUSSION

In this study we compared the angiotensin converting enzyme inhibitors captopril, enalapril and HOE 498 at concentrations which are equipotent in their effect on angiotensin I pressor response (21, 22). Their effects on the incidence and duration of ventricular fibrillation upon reperfusion after 15 min of local ischemia produced by coronary artery ligation in the isolated rat hearts were investigated. Furthermore, the influence of cyclooxygenase inhibition on the protective effects of captopril was studied. Earlier results have shown beneficial effects of captopril in this model (7, 23). These findings were confirmed in this study. HOE 498 appeared also to be effective in this model, although its beneficial effects were limited to the active, angiotensin converting enzyme inhibiting, form. No significant effects were observed for the prodrug. In contrast, enalapril did not influence arrhythmias and hemodynamics during reperfusion significantly suggesting that the mechanism is independent of the inhibition of angiotensin converting enzyme. However, enalapril did reduce purine overflow significantly during the first min of reperfusion. Therefore, it can not be excluded that other factors such as the concentration used and time to peak effect contribute to the absence of a beneficial effect of enalapril in this model.

A striking finding in the present study was the apparent association of the protection by captopril and HOE 498 against reperfusion arrhythmias and the abolishment of noradrenaline overflow

upon reperfusion. If there is a causal relationship between these events, the mechanism of the noradrenaline release and its possible modulation by converting enzyme inhibitors are important to elucidate. Local factors which might mediate the noradrenaline release observed in the control group include extracellular hyperkalemia which depolarizes the nerve endings (24). Acidosis and hypoxia have also been implicated in the direct i.e. nerve impulse independent increase in noradrenaline release from the ischemic heart. The formation of membrane active metabolites such as lysophosphoglycerides in ischemic tissue may also increase noradrenaline release secondary to depolarization of the nerve ending with concomitant influx of calcium (25). A reduced reuptake of noradrenaline has not been proposed as a major cause of depletion of myocardial stores. Reuptake however, is an active process that will be impaired if there is an insufficient supply of high energy substrates during ischemia (26); the latter is suggested by the massive overflow of ATP catabolites observed in this study in the control group. Therefore, impairment of noradrenaline reuptake still may play role in this noradrenaline overflow.

Since captopril and HOE 498 reduce the vascular overflow of noradrenaline upon reperfusion they seem to interfere with the neurogenic noradrenaline release from the presynaptic sympathetic nerve endings and not directly with the postsynaptic myocardial cells as suggested earlier (7). Several mechanisms may be involved in this effect of captopril and HOE 498 on catecholamine relase. They might act directly on presynaptic receptors which are known to have a regulatory function in the local regulation of neurotransmitter release. However,

at present only postsynaptic anti-adrenergic effects of captopril have been reported (8).

Indirectly a stimulation of prostaglandin synthesis may be involved which is supported by our findings in study B. Indomethacin abolished all the effects of captopril in our reperfusion model; reperfusion arrhythmias, purine and catecholamine overflow were again comparable to untreated hearts. There is good evidence in vivo that enhanced prostacyclin production reduces reperfusion-induced arrhythmias (31). Various other studies have pointed to prostaglandins as possible mediators of the action of converting enzyme inhibitors (27, 28) and recently a direct stimulatory action of captopril on PGI_2 synthesis in vascular tissue was shown (9). It is also known that PGI_2 inhibits noradrenaline release caused by nerve terminal depolarization in the isolated rat and rabbit heart (29). Thus, captopril and HOE 498 may reduce the catecholamine overflow indirectly via a stimulation of PGI_2 synthesis. The results of our study B indicate that this mechanism plays an important role, at least for captopril in this model since indomethacine abolishes the effect of captopril on catecholamines overflow.

Although it is known that angiotensin II facilitates adrenergic transmission by enhancing noradrenaline release upon nerve depolarization, it is unlikely that a reduction of angiotensin II concentration is involved in the observed beneficial effects. No angiotensin II appears to be produced in the isolated perfused rat heart although the presence of enzymes of the renin-angiotensin system has been demonstrated (30). In this study no angiotensin II was detected in

the coronary effluent of untreated hearts, neither during ischemia,nor during reperfusion using a sensitive radio immuno assay technique. Moreover, the lack of effect of enalapril suggests that angiotensin converting enzyme inhibition is not involved in the reduction of catecholamine overflow upon reperfusion.

In conclusion, this study shows that captopril and HOE 498 reduce the incidence and duration of malignant reperfusion arrhythmias after coronary artery occlusion in the isolated rat heart. This reduction of reperfusion arrhythmias is probably angiotensin II-independent and associated with a reduction of noradrenaline overflow. It is suggested that facilitation of prostacyclin synthesis plays an essential role in the protective effects, since indomethacine abolishes these beneficial effects for captopril.

REFERENCES

1. Corr PB, Witkowski FX. Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischaemic myocardium. Circulation 1983: 68, suppl 1: 16-24
2. Tzivoni D, Keren A, Granot H, Gottlieb S, Benhorin J, Stern S. Ventricular fibrillation caused by myocardial reperfusion in Prinzmetal's angina. Am Heart J 1983: 105: 323-325
3. Oliva PB, Breckenridge JC. Arteriographic evidence of coronary arterial apasm in acute myocardial infarction. Circulation 1977: 56: 366-374
4. Elharrar V, Zipes DP. Cardiac electrophysiologic alterations during myocardial ischemia. Am J Physiol 1977: 232: H329-H345
5. Manning AS, Hearse DJ. Reperfusion-induced arrhythmias: mechanisms and prevention. J Mol Cell Cardiol 1984: 16: 497-518
6. Hearse DJ. Critical distinctions in the modification of myocardial cell injury. In: Opie LH (ed) Calcium Antagonists. New York: Raven Press, 1983.
7. Gilst WH van, Graeff PA de, Kingma JH, Wesseling H, Langen CDJ de. Captopril reduces purine loss and reperfusion arrhythmias in the rat heart after coronary artery occlusion. Eur J Pharmacol 1984: 100: 113-117
8. Saruta T, Suzuki H, Okundo T, Kondo K. Effects of angiotensin-converting enzyme inhibitors on the vascular response to norepinephrine. Am J Cardiol 1982: 49: 1535-1536

9. Düsing R, Scherag R, Landsberg G, Glanzer K, Kramer HJ.The converting enzyme inhibitor captopril stimulates prostacyclin synthesis by isolated rat aorta. Eur J Pharmacol 1983: 91: 501-504

10. Koomen JM, Gilst WH van, Zimmerman ANE, Noordwijk J van. A concentration-dependent biphasic positive inotropic action of ouabain on isolated hearts of rat and guinea-pig. Arch Int Pharmacodyn Ther 1982: 255: 2

11. Kannengieser GJ, Lubbe WF, Opie LH. Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. J Mol Cell Cardiol 1975: 7: 135-151

12. Lubbe WF, Daries PS, Opie LH. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc Res 1978: 12: 212-220

13. Gilst WH van, Langen CDJ de. Ischemia-reperfusion induced arrhythmias in the isolated rat heart. Pharm Weekblad Sci ed 1982: 45: 160

14. Stam H, Jong JW de. Sephadex-induced reduction of coronary flow in the isolated rat heart: a model for ischemic heart disease. J Mol Cell Cardiol 1977: 9: 633

15. Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRM, Langen CDJ de. Improved functional recovery of the isolated rat heart after 24 hours of hypothermic arrest with a stable prostacyclin analogue (ZK 36 374). J Mol Cell Cardiol 1983: 15: 789-792

16. Gobel FL, Nordstrom LA, Nelson RR, Jorgenson CR, Wang Y. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 1978: 57: 549-556

17. Neely JR, Whitmer KM, Mochizuki S. Effects of mechanical activity and hormones on myocardial glucose and fatty acid utilization. Circ Res 1976: 38 (I): 22 - 30.

18. Jong JW de, Verdouw PD, Remme WJ. Myocardial nucleoside and carbohydrate metabolism and hemodynamics during partial occlusion and reperfusion of pig coronary artery. J Mol Cell Cardiol 1977: 9: 297

19. Harmsen E, Jong JW de, Serruys PW. Hypoxanthine production by ischemic heart demonstrated by high performance liquid chromatography of blood purine nucleosides and oxypurines. Clin Chim Acta 1981: 115: 73

20. Westerink BHC. Analysis of trace amounts of catecholamines and related compounds in brain tissue: a study near the detection limit of liquid chromatography with electrochemical detection. J Liquid Chrom 1983: 6, 12: 2337-2351

21. Gross DM, Sweet CS, Ulm EH, Backlund EP, Morris AA, Weitz D, Bohn DL, Wenger HC, Vassil TC, Stone CA. Effect of N- (S)-1-carboxy-3-phenylpropyl-L-ala-L-pro and its ethyl ester (MK-421) on angiotensin converting enzyme in vitro and angiotensin I pressor responses in vivo. J Pharm Exp Ther 1980: 216: 552-558

22. Becker RHA, Schölkens BA, Metzger M, Schulze KJ. Pharmacological properties of the new orally active converting enzyme inhibitor HOE 498. Drug Research 1984: 34 (II): 1411-1416

23. Graeff PA de, Gilst WH van, Langen CDJ de, Wesseling H. Concentration-dependent protection by captopril against reperfusion injury in the isolated rat heart. Circulation 1984: 70: 4, II-89

24. Borda L, Schuchleib R, Henry PD. Effects of potassium on isolated canine coronary arteries: modulation of adrenergic responsiveness and release of norepinephrine. Circ Res 1977: 14: 778-786

25. Corr PB, Snyder DW, Lee BI, Gross RW, Keim CR, Sobel BE. Pathophysiological concentrations of lysophosphatides and the slow response. Am J Physiol 1982: 243: 187-195

26. Wakade AR, Furchgott RF. Metabolic requirements for the uptake and storage of norepinephrine by the isolated left atrium of the guinea-pig. J Pharmacol Exp Ther 1968: 163: 123-135

27. Swartz SL, Williams GH, Hollenberg NK, Levine L, Dluhy RG, Moore, FJ. Captopril-induced changes in prostaglandin production. J Clin Invest 1980: 65: 1257

28. Witzgall H, Hirsch F, Scherer B, Weber PC. Acute haemodynamic and hormonal effects of captopril are diminished by indomethacin. Clin Sci 1982: 62: 611

29. Khan MT, Malik KU. Modulation by prostaglandins of the release of H3 noradrenaline evoked by potassium and nerve stimulation in the isolated rat heart. Eur J Pharmacol 1982: 78: 213-218

30. Ganten D, Schelling P, Vescei P, Ganten U. Iso-renin of extrarenal origin. The tissue angiotensinogenase systems. Am J Med 1976; 60: 760-772

31. Coker SJ, Parratt JR. The effects of dazoxiben on arrhythmias and ventricular fibrillation induced by coronary artery occlusion and reperfusion. Br J Clin Pharmacol 1983 ; 15 (suppl 1):87S-95S.

APPENDIX V

CALCIUM REPLETION-INDUCED ARRHYTHMIAS AFTER SHORT PERIODS OF CALCIUM-FREE PERFUSION IN THE ISOLATED RAT HEART

W.H. van Gilst and J.M. Koomen

Submitted for publication in

Basic Research in Cardiology

CALCIUM REPLETION-INDUCED ARRHYTHMIAS AFTER SHORT PERIODS OF CALCIUM-FREE PERFUSION IN THE ISOLATED RAT HEART

SUMMARY

The role of calcium ions in the genesis of arrhythmias was studied in the "calcium-paradox" model in the isolated rat heart. Clear relationships were found between the duration of calcium-free perfusion and (a) the occurrence of calcium-free-induced electrophysiological changes, (b) the incidence and duration of subsequently induced calcium-repletion arrhythmias and (c) mechanical recovery at the end of the repletion period.

The first signs of electrophysiological changes (i.e. decreased heart rate, T-wave amplitude and increased PQ-interval) and irreversible loss of myocardial recovery occurred during or after 60-90 s of calcium-free perfusion. The occurrence of calcium-repletion induced ventricular tachycardia parallelled this onset of irreversible cardiac injury. These results suggest that the process of calcium washout and subsequent sudden calcium overloading may play a role as trigger in the pathogenesis of ventricular arrhythmias.

INTRODUCTION

The incidence and characteristics of arrhythmias induced by reperfusion of the previously ischemic myocardium are extensively studied (1,12,13,14). However, the precise electrophysiological mechanisms underlying the genesis of these arrhythmias remain unclear. Beside other factors a loss of control of intracellular calcium homeostasis has been associated with many aspects of tissue injury during ischemia as well as reperfusion (2,6,15). It has been suggested (3) that an increase in calcium uptake during reperfusion may cause delayed after-depolarizations and in this way account for an increase in idioventricular rate and ventricular arrhythmias. However, since ischemia leads to a complex alteration of ionic transport mechanisms and metabolic processes in the myocardial cell (3,15,16), it is difficult to conclude from experimental ischemia-reperfusion models whether reperfusion-arrhythmias are exclusively triggered by intracellular calcium overloading or that other factors are essentially involved in this pathogenic process.

In order to study the role of calcium overloading in the pathogenesis of arrhythmias we used the "calcium paradox"-model (19). Many investigators have shown that this is a useful experimental model to study the effects of myocardial calcium overload. During the calcium-free as well as the calcium-repletion period cardiac electrical activity was studied and compared with contractile recovery at the end of the repletion period.

METHODS

Male Wistar rats (225-275 g) were anaesthetized with diethylether. After heparinization via the tailvein the thorax was opened, the aorta cannulated, the heart quickly removed and perfused according to the method of Langendorff at a constant perfusion pressure of 8 kPa and a temperature of 37°C. The mitral valve of the left heart chamber was dissected in order to avoid ventricular wall distension by fluid accumulation. Standard salt solution contained respectively (mM): NaCl 129.20; KCl 4.70; $CaCl_2$ 1.36; $MgCl_2$ 1.15; NaH_3PO_4 0.41; $NaHCO_3$ 20.20; glucose 11.10. Calcium-free perfusion buffer was obtained by omitting $CaCl_2$ from the standard perfusion buffer. No corrections were made for the small change in osmolarity due to the omission of CaCl and no EDTA was added in order to avoid secundary effects of EDTA per se. After saturation of the perfusion media with carbogen (95% O_2 + 5% CO_2) pH of the solutions was 7.35 ± 0.05.

Mechanical functioning of the isolated heart was measured by recording apico-basal shortening of the heart isotonically with a displacement transducer (HBM-W10). Changes in cardiac resting length were expressed as absolute changes in apico-basal length during diastole with respect to resting length obtained at the end of the stabilization period.

The electrocardiogram and frequency of heart pulses were derived

directly from the heart via the stainless steel aortic cannula and a Pt-Ir-electrode hooked into the left ventricular wall at a fixed point near the apex of the heart.

ECG was stored on magnetic tape (AMPEX FR1300) during the whole experiment. Heart rate, PQ-interval and the occurence of arrhythmias were evaluated with the CENSOR computer program (18) on a PDP 11/45 computer.

Coronary flow rate was measured continuously throughout the experiment with an electromagnetic flow meter (Skala MDL-400) placed just above the aortic canula.

The following perfusion sequence was employed. After a stabilization period of 30 min seven groups of six hearts were perfused instantaneously with calcium-free perfusion buffer for a fixed period of 30, 60, 75, 90, 120, 150 and 480 seconds, respectively, in order to assess the nature and time-course of the initial changes in cardiac electrical activity during calcium depletion. Changes in cardiac electromechanical functioning due to calcium repletion were studied during and at the end of a subsequent normal, calcium containing, repletion period (recovery phase) of 30 min.

The results are presented as the mean $\pm$ SEM of six experiments. Tests of significance were calculated by Student's t-test ($p < 0.05$).

RESULTS

Effects of calcium-free perfusion.

Calcium-free perfusion of the isolated rat heart resulted in a rapid decline in contractile activity . Contracture development (= decrease in resting length) started after 30-60 s of calcium-free perfusion and reached a plateau-value after 90 s of calcium-free perfusion (Fig. 1). Coronary flow increased markedly during cessation of

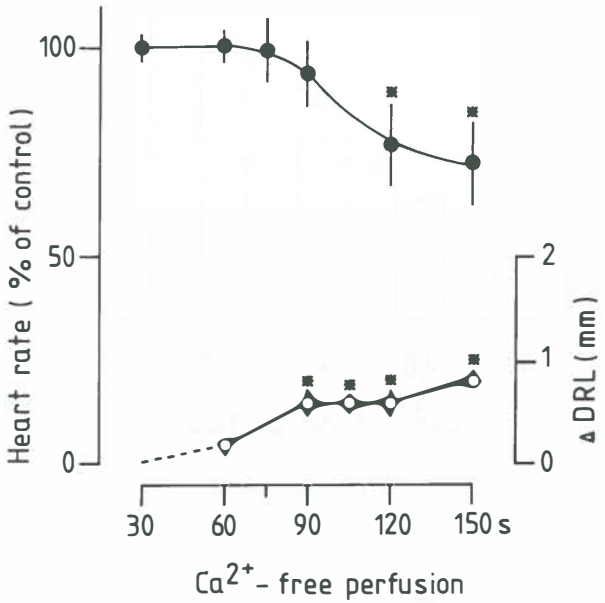

Fig. 1. Changes in heart rate (●—●) and diastolic resting length (DRL, o—o) following different durations of calcium-free perfusion. Results are means ± S.E.M. of 6 observations at the end of the calcium-free perfusion period.

*p < 0.05 versus values at the end of the control perfusion period.

contractions and maintained elevated at a plateau-value during the rest of the calcium-free perfusion period. There appeared to be a linear relationship between the duration of the calcium-free period and the total amount of calcium-free perfusate which passes through the heart during this period (Fig. 2), thus calcium washout seemed to be a linear process (13).

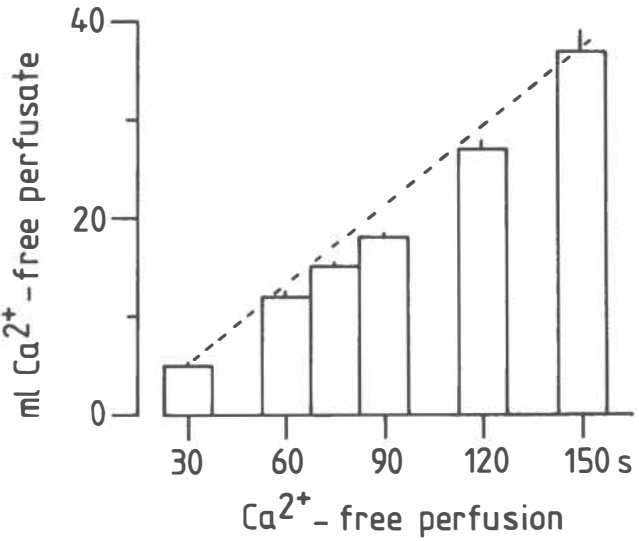

Fig. 2. Relationship between the duration of the calcium-free period and the total amount of calcium-free perfusate which passes through the heart during this period. Results are the mean ± S.E.M. of 6 observations.

Besides a slight decrease in QRS-amplitude and T-wave, the complex form in the electrocardiogram showed only minor changes during calcium-free perfusion. However, heart rate was significantly reduced after 120 s calcium-free perfusion (Fig. 1). In addition AV nodal conduction time decreased significantly when the duration of the calcium-free perfusion was 60 s or longer as can be seen in Fig. 3 in which the PQ-intervals at the end of the calcium-free perfusion period are indicated.

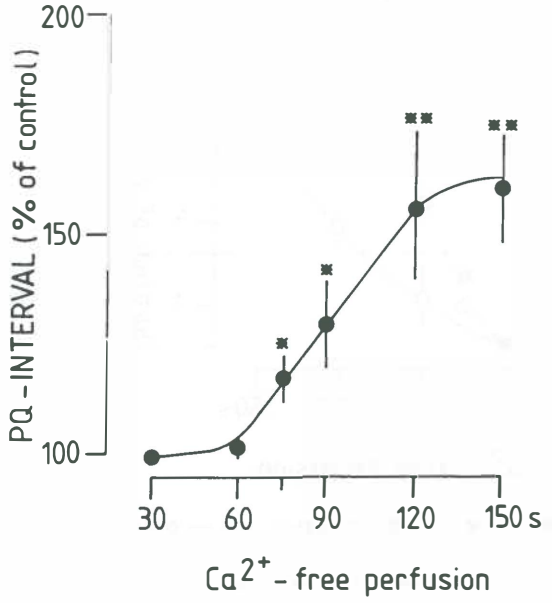

Fig. 3. Changes in AV conduction (PQ interval) following different durations of calcium-free perfusion. Results are mean ± S.E.M. of 6 observations at the end of the calcium-free perfusion period.
*p < 0.05 **p < 0.005 versus PQ interval values at the end of the control perfusion period.

Calcium-repletion period.

Upon normal, calcium containing, reperfusion rapid changes in cardiac electrical activity were observed, dependent on the duration of the preceding calcium-free period. After 75 s of calcium-free perfusion

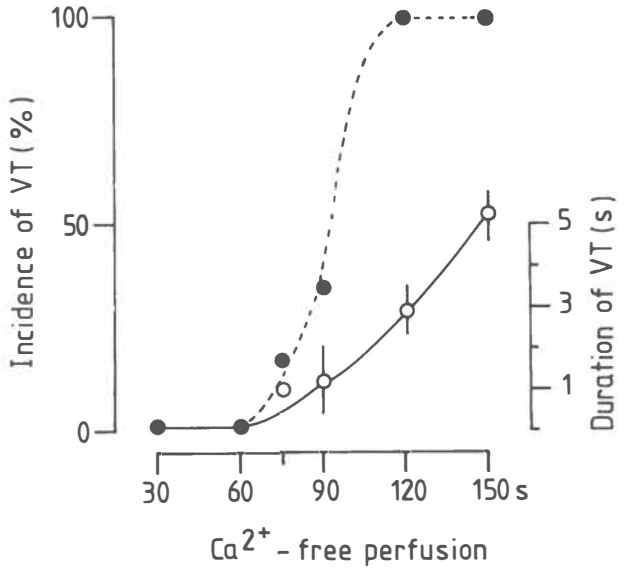

Fig. 4. Incidence (● —— ●) and duration (o — o) of ventri-cular tachycardia upon normal, calcium containing, reperfusion following different durations of calcium-free perfusion.

hearts started to show a short period of ventricular tachycardia upon calcium-repletion (Fig. 4). The incidence and duration of these ventricular tachycardias rapidly increased with increasing duration of calcium-free perfusion (Fig. 4). After 120 s of calcium-free perfusion

this period of ventricular tachycardia was followed by a complete disappearence of electrical activity of the ventricles and only a reduced atrial activity could be registrated (Fig. 5). These hearts only showed a slight recovery of electrical activity at the end of the repletion period with ventricular depolarizations at a very low rate (30 bpm).

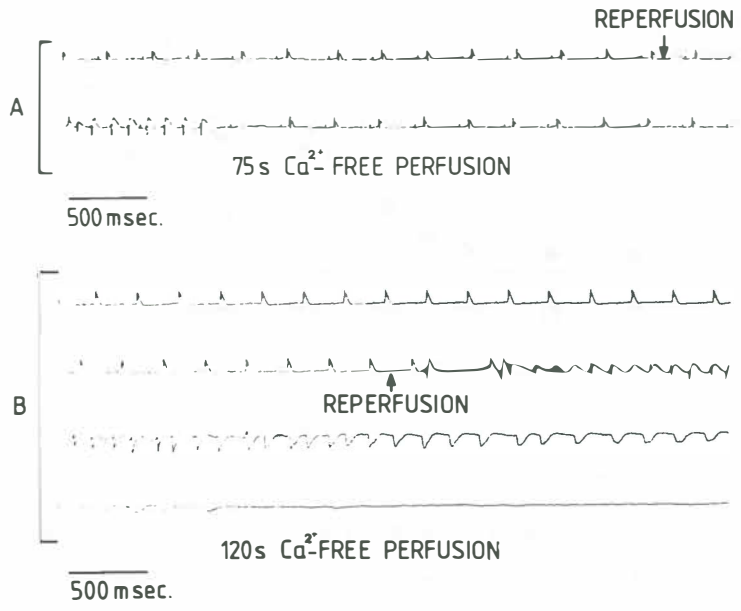

Fig. 5. Typical recordings of ventricular tachycardias occuring upon calcium-reperfusion after a period of (a) 75 s and (b) 120 s calcium-free perfusion.

Recovery of mechanical activity at the end of the repletion period also was dependent on duration of calcium-free perfusion period and parallelled the observed changes in electrical activity. After 75 s of

calcium-free perfusion a rapid decline in recovery of contraction

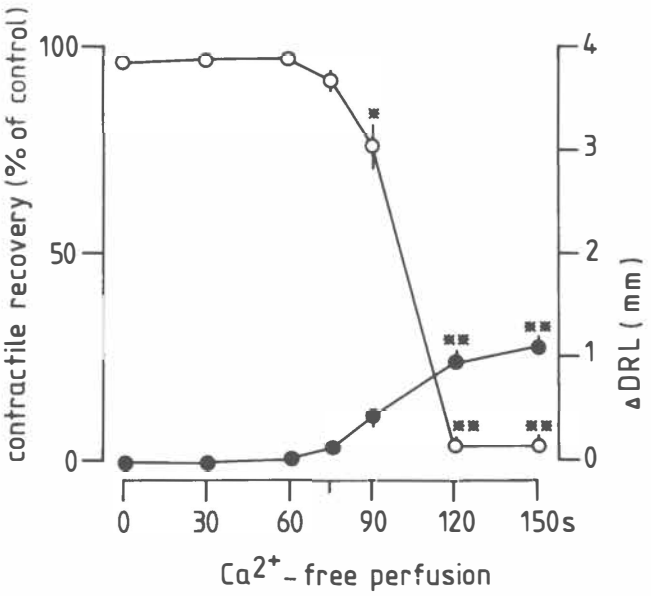

Fig. 6. Contractile recovery (o —o) and changes in diastolic resting length (DRL, ●—●) following different durations of calcium-free perfusion. Results are means ± S.E.M. of 6 observations at the end of the reperfusion period. *p < 0.05 **p < 0.005 versus values at the end of the control perfusion period.

amplitude was observed (Fig. 6). A concomitant contracture development was also manifest during calcium-repletion (Fig. 6). Recovery of coronary flow plotted against duration of calcium-free perfusion appeared to be a bell shape curve (Fig. 7), with a peak in coronary flow after 120 s calcium-free perfusion.

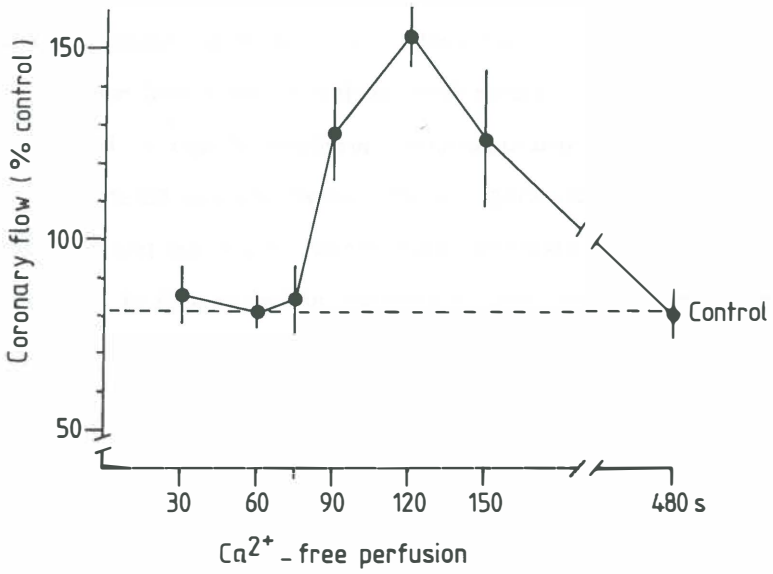

Fig. 7. Recovery of coronary flow following different durations of calcium-free perfusion. Results are means ± SEM of 6 observations at the end of the reperfusion period.

DISCUSSION

The results from this study show clear relationships between the duration of calcium-free perfusion and (a) the occurrence of calcium-free-induced electrophysiological changes (see figure 1 and 3), (b) the incidence and duration of subsequently induced calcium-repletion arrhythmias (figure 4) and (c) mechanical recovery at the end of the repletion period (figure 6). The first signs of electrophysiologic disturbances and irreversible loss of mechanical recovery (i.e. during

calcium-repletion) always occurred during (figure 1 and 3) and after 60-90 s of calcium-free perfusion. The functional changes observed during the period of calcium-free perfusion (decreased heart rate, increased PQ-interval and contracture development) have to be a direct consequence of the (advancing) calcium-washout process. Several authors have suggested that even very short periods of calcium-free perfusion may result in a rapid and irreversible loss of control of cellular calcium homeostasis due to disturbances in cell membrane regulatory functions (7,9,19).

The occurrence of calcium-repletion arrhythmias (i.e. ventricular tachycardia; see figure 4 and 5) parallelled the onset of irreversible cardiac injury as was reflected by an incomplete recovery of myocardial contractility (see figure 6). It has been demonstrated by many authors that calcium-reperfusion-induced irreversible cardiac damage is a consequence of intracellular calcium overload (7,8). The parallellism we found between the loss of mechanical function and the incidence and duration of ventricular arrhythmias during calcium-reperfusion makes it tempting to suggest that calcium overloading (uncontrolled calcium uptake) is related to the generation of these arrhythmias. The precise role of calcium in the genesis of calcium-repletion injury, however, remains to be clarified. Attempts to reduce the excessive uptake of calcium ions during calcium-repletion as well as during post-ischemic reperfusion by blocking the voltage-dependent calcium channels with calciumantagonist only delivered very conflicting results thus far (13).

Like ischemia-induced arrhythmias, reperfusion arrhythmias can be

divided into different subsets according to their appearance, time-dependent occurrence and proposed mechanism of origin (13). In the case of 'early' reperfusion arrhythmias heterogeneity of rapidly changing cellular processes may be of crucial importance in the determination of the extent of electrical instability (12). The rapid cellular uptake of calcium upon repletion may enhance the heterogeneity in cellular injury due to ischemia and, in our model, to calcium-free perfusion. The multiformity of the calcium-repletion induced tachycardias we observed on our ECG-tracings suggests a continuous change in wave front pathway or a changing site of focal activity. We suggest that this heterogeneity may be induced by the heterogenous process of the actual calcium-washout during the calcium-free period as well as a heterogeneity of the calcium-uptake process during subsequent calcium-repletion. In hearts which underwent 75 s or less calcium free perfusion the ventricular tachycardias observed upon calcium-repletion were followed by a sudden return of sinus rhythm (Fig. 5). This observation suggests that the onset of the process of calcium uptake itself is arrhythmogenic and not the end result of calcium uptake, i.e. irreversible cellular damage.

Another interesting result of this study is the absence of ventricular fibrillation during as well as after calcium-free perfusion in all experiments. This result is in contrast with the high incidence of ventricular fibrillation observed during as well as after ischemia in the isolated perfused mammalian hearts (3,5,12,17).The absence of ventricular fibrillation, also reported by other investigators (4), may be explained as follows. Corr and Witkowski (3) suggested that during ischemia the composition of the perfusate, which a.o. depends upon the

accumulation of toxic metabolites, might profoundly alter the ultimate vulnerability of cardiac tissue to the induction of arrhythmias. There is no accumulation of these substances in our model. Thus, as was also suggested by Manning and Hearse (13), the heterogeneity during calcium-repletion is probably less severe and complex as during post-ischemic reperfusion. Janse and Kleber (10) suggested that in the ischemic myocardium macro-reentrant circuits are responsible for ventricular tachycardia, whereas degeneration of these macro-circuits into micro-reentrant circuits will result in subsequent ventricular fibrillation. We suggest that calcium-repletion may induce macro-reentry circuits and thus ventricular trachycardia, but is unable to induce micro-reentry circuits and therefore fails to induce ventricular fibrillation.

In conclusion, the data obtained from this study make an involvement of the process of calcium overloading in the induction of calcium-repletion-induced ventricular tachycardias very likely and show that the calcium-repletion-induced electrical disturbances are narrowly related to the ultimate loss of mechanical activity and development of contracture during normal recovery after calcium-free perfusion.

REFERENCES

1. Braunwald E, Kloner RA (1982) The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 66:1146-1149.
2. Clusin WT, Buchbinder M, Harrison DC (1983) Calcium overload, 'injury' current, and early ischaemic cardiac arrhythmias - a direct connection. Lancet 17:272- 273.
3. Corr PB, Witkowski FK (1983) Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischaemic myocardium. Circulation 68, Suppl. 1:16-24.
4. Capucci A, Janse MJ, Ruigrok TJC (1983) The calcium paradox: an electrophysiological study in the isolated rabbit heart. Eur. Heart J. 4, Suppl. H:13-21.
5. Gilst WH van, Graeff PA de, Kingma JH, Wesseling H, Langen CDJ de (1984) Captopril reduces purine loss and reperfusion arrhythmias in the rat heart after coronary artery occlusion. Eur. J. Pharmacol. 100:113-117.
6. Grinwald PM (1982) Calcium uptake during post-ischemic reperfusion in the isolated rat heart: influence of extracellular sodium. J. Mol. Cell. Cardiol. 14:359-365.
7. Grinwald PM, Nayler WG (1981) Calcium entry in the calcium paradox. J. Mol. Cell. Cardiol. 13:867-880.
8. Hearse DJ, Baker JE, Humphrey SM (1980) Verapamil and the calcium paradox. J. Mol. Cell. Cardiol. 12:733-739.
9. Holland CE, Olson RE (1975) Prevention by hypothermia of paradoxical necrosis in cardiac muscle. J. Mol. Cell. Cardiol. 7:917-928.

10. Janse MJ, Kleber AG (1981) Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischemia. Circ. Res. 49: 1069-1081.

11. Koomen JM et al. (1983) Myocardial recovery from global ischemia and reperfusion: Effects of pre- and/or postischemic perfusion with low-calcium. J. Mol. Cell. Cardiol. 15:383-392.

12. Lubbe WF, Daries DS, Opie LH (1978) Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc. Res. 12:212-218.

13. Manning AS, Hearse DJ (1984) Reperfusion-induced arrhythmias: Mechanisms and prevention. J. Mol. Cell. Cardiol. 16:497-518.

14. Murdock DK, Loeb JM, Euler DE, Randall WC (1980) Electrophysiology of coronary reperfusion. Circulation 61:175-182.

15. Nayler WG (1981) The role of calcium in the ischemic myocardium. Am. J. Pathol. 102:262-270.

16. Opie LH, Nathan D, Lubbe WF (1979) Biochemical aspects of arrhythmogenesis and ventricular fibrillation. Am. J. Cardiol. 43:131-148.

17. Penny WJ, Sheridan DJ (1983) Arrhythmias and cellular electrophysiological changes during myocardial "ischaemia" and reperfusion. Cardiovasc. Res. 17:363-272.

18. Swenne CA, Van Hemel NM (1983) Algorithms for the interpretation of ventricular arrhythmias. In: Proc Computers in Cardiol. Silver Spring, MD, IEEE Computer Society Press 231-241.

19. Zimmerman ANE, Hülsmann WC (1967) Paradoxical influence of calcium ions on the permeability of the cell membranes of the isolated rat heart. Nature 211:646-647.

APPENDIX VI

EFFECTS OF DILTIAZEM ON ISCHEMIA- AND REPERFUSION-INDUCED MYOCARDIAL INJURY IN VITRO AND IN VIVO

W.H. van Gilst, P.A. de Graeff, J.H. Kingma,
C.D.J. de Langen and H. Wesseling

Submitted for publication in the
Journal of Molecular and Cellular Cardiology

EFFECTS OF DILTIAZEM ON ISCHEMIA- AND REPERFUSION-INDUCED MYOCARDIAL INJURY IN VITRO AND IN VIVO

SUMMARY

The effects of the calcium antagonist, diltiazem, on myocardial injury during ischemia and reperfusion were studied both in vitro, in the isolated rat heart, and in vivo, in a closed-chest pig model. In the isolated rat heart, administration of diltiazem before or at the onset of ischemia resulted in a dose-dependent reduction of the incidence and duration of ventricular fibrillation. This reduction was associated with a dose-dependent reduction in overflow of ATP catabolites (adenosine, inosine, hypoxanthine and xanthine). Both changes were significant at concentrations of 0.3 μM diltiazem and higher. When 0.3 μM diltiazem was administered upon reperfusion no effect on the incidence of ventricular fibrillation and on ischemia induced total purine overflow was observed. However, the duration of ventricular fibrillation and purine overflow at 5 min after reperfusion were significantly reduced.

In the pig experiments all untreated animals(n=8) showed accelerated idioventricular rhythm (AIVR) upon reperfusion which lasted for 22 $\pm$ 5 min after which sinus rhythm returned. Only two out of five treated animals (450 μg/kg/2h) had an AIVR for 2 resp. 8 min. Upon reperfusion both groups showed a substantial rise in noradrenaline concentration in the coronary sinus blood, but after 5 min this was significantly less in the treated group. Creatine kinase-kinetics were

not altered by diltiazem, but the maximum creatine kinase level was significantly reduced. Within 4 days after the acute experiment 50% of the untreated animals died suddenly, whereas no sudden deaths occurred in the diltiazem group ($p < 0.05$). Seven days after the acute experiment, sustained ventricular tachycardia could be induced with programmed electrical stimulation in 3 out of 4 surviving untreated pigs. In none of the diltiazem treated pigs ventricular tachycardia was inducible.

The results of this study show that the calcium antagonist diltiazem can beneficially influence the events during ischemia and during reperfusion, both in vitro and in vivo; this is associated with a reduction of ATP catabolism, creatine kinase release and noradrenaline overflow. Furthermore, diltiazem reduces electrical instability in the chronic phase of myocardial infarction.

INTRODUCTION

Myocardial ischemia is known to produce cardiac injury (10,20) and is associated with ventricular arrhythmias (1,8,42). When the initial ischemic period is of sufficient duration, the return of flow can further exacerbate tissue damage (9,19,45) and provoke malignant ventricular arrhythmias (48), which appear to differ in underlying mechanism from ischemia-induced arrhythmias (1,6,25,32,33,42). These sequences of events have been suggested to be a major cause of sudden cardiac death in man (41).

The mechanism(s) responsible for this paradoxical extension of injury is unknown but there is some evidence that it may involve a defect in calcium homeostasis (21,37). Based on this hypothesis the effects of various calcium channel blocking drugs on ischemia and reperfusion induced phenomena have been investigated (37,44,53). However, despite their increasing use in the treatment of ischemic heart disease, the efficacy of these drugs in a variety of different models of ischemia and reperfusion remains a matter of debate (11,26,36,50). Since the main action of calcium antagonists is an inhibition of slow channels not only in myocardial cells but also in smooth muscle cells (which results in a systemic and coronary vasodilation), it is difficult in intact animals to distinguish the beneficial effect of improved oxygen supply-demand ratio from favorably influenced myocardial metabolism, such as ATP sparing effects (22) or prevention of calcium overloading (36).

Therefore, we examined the effects of the calcium antagonist diltiazem on myocardial function during ischemia and/or reperfusion both in vitro in an isolated rat heart model and in vivo in a closed chest pig model.

MATERIALS AND METHODS

A. Isolated rat heart model

Male Wistar rats (275-325 g), fed ad libitum, were anesthetized with ether and given 500 I.U. of heparin intravenously. The hearts were rapidly excised and arrested in icy-cold 0.9% NaCl. Retrograde perfusion of the aorta as described by Langendorff was immediately started with a modified Tyrode solution (28), containing 10 mM glucose and equilibrated with 95% 0_2 + 5% CO_2. This perfusion buffer was filtered through 1.2 μM pore size filters before reaching the heart. The perfusion pressure was maintained at 60 mmHg. The temperature was continuously measured in the aorta-cannula tip and kept between 36.5 and 37.5°C. The hearts beated spontaneously.

Acute regional myocardial ischemia was produced as described by Kannengieser et al. (24). The left main coronary artery was ligated with 6-0 silk 2 mm below the aortic root using a 3/8 circle taper point needle (Ethicon). Reperfusion of the ischemic tissue was achieved by releasing the ligation. This technique of reversible ligation of the

left coronary artery has proven to be a useful model to provoke ventricular arrhythmias (12,31).

Measurement of mechanical and electrophysiological parameters

The pressure-rate product was used as an index of contractility (13,47) and hence of oxygen consumption (15,40). Left ventricular end systolic pressure (LVP) was measured by means of a catheter inserted into the left ventricle via the mitral valve and connected to a pressure transducer (Statham P23 Db). Pressure-rate index was calculated as the product of maximal LVP and heart rate, and expressed as percentages of the values at the end of the equilibration period.

A cardiac electrogram was obtained by means of two electrodes: one attached to the metal inflow cannula and the other to the ventricular apex outside the ischemic zone. Heart rate, PQ interval and the occurrence of arrhythmias were monitored by continuous registration of the cardiac electrogram. The ECG was visualised using an ink jet recorder at a paper speed of 100 mm/s (Siemens Oscillomink E).

Coronary flow (volume of perfusion fluid per time unit) was measured by a microprocessor, which controlled the perfusion pressure by adjusting the peristaltic perfusion pump (LKB, microperpex).

Assay of ATP catabolites

Overflow of adenosine and its catabolites (inosine, hypoxanthine and xanthine) was measured as an indicator of nucleotide breakdown. This has proven to be a reliable parameter for the degree of ischemia-induced

cellular damage (14,23). The purine nucleosides and oxypurines were determined by a slightly modified version of the high-performance liquid chromatography assay described by Harmsen et al. (17). At the end of the experiment, the hearts were dried to constant weight and the purine overflow was expressed as nmol/min g dry weight (g dwt).

Protocol

The hearts were allowed to equilibrate with the perfusion fluid for 15 min. After this equilibration period and a control perfusion of 15 min, local ischemia was induced and maintained for the next 15 min. After reperfusion of the ischemic zone for 30 min the experiments were terminated.

The rat hearts were divided at random into 8 groups. In five groups diltiazem was added at the start of the control period and treatment was continued during the whole experiment, including the period of reperfusion. Drug concentrations in these groups were O.03 (n=6), 0.1 (n=6), 0.3 (n=8), 1 (n=7) and 3 μM (n=4) diltiazem respectively. In one group (n=7) diltiazem was added at the onset of ischemia and in another group (n=6) at the beginning of reperfusion. Diltiazem concentration in these groups was 0.3 μM, since pilot studies had shown that with this concentration the first significant effect on the pressure-rate index was obtained. One group served as control (n=15).

B. Closed-chest pig model

Acute experiment

Male Yorkshire swine (body weight, 25-35 kg) were pretreated with 120 mg azaperone (Stresnil; Janssen Pharmaceutica Beerse, Belgium) intramuscularly. Half an hour later 150 mg metomidate (Hypnodil; Janssen) was injected in an ear vein. A cuffed endotracheal tube was introduced and the animals were ventilated with a mixture of O_2/N_2O. Anesthesia was maintained with an intravenous infusion of azaperone (2 mg/kg/min) and metomidate (8 mg/kg/min) through a double-lumen catheter in the inferior vena cava. Ventilation parameters were adjusted to keep arterial PCO_2 concentrations between 4.5 and 6.5 kPa and PO_2 concentrations between 16 and 20 kPa. Body temperature was kept at 36-37°C with a thermal mattres. Arterial blood pressure was monitored with a catheter in a femoral artery. Blood samples taken from this catheter were used for monitoring creatine kinase and blood gas levels. Left ventricular oxygen consumption was estimated by calculation of the product of mean arterial pressure and heart rate (15,40). Cardiac output was monitored by thermodilution with a Swan-Ganz catheter advanced through the external jugalar vein. Heparin was administered at an initial dose of 5000 IU, followed by 2500 IU/h. A polyethylene catheter was introduced in the coronary sinus via the left jugular vein for monitoring catecholamine overflow. Analysis of catecholamines was based on high-performance liquid chromatography and electrochemical detection as described by Westerink (51).

A modified 7 French Sones catheter was introduced via the left carotid artery and the tip was positioned at the ostium of the left coronary artery as verified by fluoroscopy. A balloon catheter as used for percutaneous coronary angioplasty (PTCA) was introduced through the lumen of the Sones guiding catheter and positioned in the anterior descending branch of the left coronary artery and advanced beyond the first diagonal artery. In order to obtain reproducible ischemic regions the balloon position was carefully checked by contrast fluoroscopy.

After positioning of the catheters animals were allowed to equilibrate till stable baseline values were observed. After this equilibration period and a control period of 30 min, ischemia was induced by inflating the balloon during 60 min. A bolus injection of lidocaine (1 mg/kg) was administered 2 min before ischemia. After reperfusion of the ischemic zone for 90 min the experiments were terminated. After removal of the catheters pigs were subjected to routine post-operative care and returned to their cages.

Five pigs received diltiazem during the acute experiment. Treated animals were infused during the first 10 min of the control period with 300 μg/kg diltiazem and they received 150 μg/kg diltiazem during the subsequent 110 min. Eight pigs served as control.

Programmed electrical stimulation one week after the acute experiment

Seven days after the occlusion/reperfusion procedure programmed electrical stimulation of the heart was performed in the surviving

animals, to evaluate the inducibility of ventricular tachycardia or -fibrillation. Induction and maintainance of anaesthesia were the same as described before. Positioning of electrode catheters and stimulation protocol were performed as described earlier (29). Stimuli were delivered at twice diastolic threshold. The stimulation protocol included stimulation the RVA during sinus rhythm and at 3 different cycle lengths, 600, 500 and 430 msec, with single, double and triple stimuli.

Animals were regarded inducible when a sustained VT or VF occurred during programmed electrical stimulation. A tachycardia was defined as sustained when it lasted more than 30 sec and as non-sustained if there were at least 6 non stimulated beats and it lasted not longer than 30 sec. Distinction between a supra- and ventricular origin of the tachycardia was made by use of the His bundle electrogram.

Four surface (Einthoven leads I, II, III, and one unipolar midchest lead) electrocardiograms and three intracardiac electrograms (HRA, HIS and RVA) were recorded on magnetic tape (Hewlett-Packard 3968) and registered on a Siemens Mingograf ink-jet recorder at a paper speed of 100 mm/s.

After the electrophysiologic programmed electrical stimulation study, the animals were sacrificed and their hearts were excised and analyzed macroscopically for the localization and extent of infarcted tissue.

Reagents

All chemicals were analytical grade. Diltiazem was a gift of Lorex Pharmaceutica B.V. Fresh solutions were prepared daily.

Statistical analysis

The data are expressed as the means ± SEM. Group differences were tested with Student's t-test, Mann-Whitney's U-test or Fisher's exact probability test; p values less than 0.05 double-sided were considered to be significant.

RESULTS

A. Isolated rat heart model

Diltiazem present throughout the whole experiment

Fig. 1A shows the dose-dependent effects of diltiazem on coronary flow and pressure-rate index at the end of the control perfusion period (t = 15). Interestingly, a significant increase in coronary flow was observed at a concentration 10 fold higher than the concentration which produced a significant decrease in pressure-rate index.

Occlusion of the left coronary artery resulted in an average decrease of total coronary flow of 44 ± 4% SEM for all groups. Pressure-rate index was severely impaired for all groups during ischemia and no significant differences in pressure-rate index were observed between the groups (Table I). However, a dose-dependent reduction of purine overflow was observed after 15 min of ischemia (Table I).

Release of the ligation resulted in a rapid and total recovery of coronary flow for all groups. Upon reperfusion, all untreated hearts fibrillated (Fig. 1B). This ventricular fibrillation was self terminating and lasted for 13.2 ± 2.0 min. In the presence of diltiazem, both incidence and duration of this reperfusion-induced ventricular fibrillation were reduced in a dose-dependent manner.

Release of the coronary ligation resulted in a substantial overflow of ATP catabolites for all groups (Fig. 1C). This purine overflow was dose-dependently reduced by diltiazem and significant at 0.3 μM and

higher concentrations. At the end of the reperfusion period this reduced

Table 1. Effect of diltiazem on the isolated rat heart at the end of the ischemic period. Treatment started at t = 0.

	Diltiazem (M)					
	Untreated	3.10^{-8}	10^{-7}	3.10^{-7}	10^{-6}	3.10^{-6}
Coronary flow (% of t = 0)	53 ± 6	58 ± 3	53 ± 5	66 ± 6	69 ± 8	82 ± 6*
Heart rate (% of t = 0)	91 ± 3	99 ± 5	78 ± 4	74 ± 5*	54 ± 4*	51 ± 3*
Pressure-rate index (% of t = 0)	34 ± 5	37 ± 4	26 ± 4	24 ± 5	24 ± 6	24 ± 6
Purine overflow (nmol/min.gdwt)	102 ± 10	112 ± 9	129 ± 14	55 ± 9*	42 ± 10*	20 ± 4*

values are mean ± SEM; * indicates a significant change ($p < 0.05$) when compared to control.

Effect of diltiazem on the isolated rat heart at the end of the reperfusion period. Treatment started at t = 0.

	Diltiazem (M)					
	Untreated	3.10^{-8}	10^{-7}	3.10^{-7}	10^{-6}	3.10^{-6}
Coronary flow (% of t = 0)	75 ± 5	76 ± 4	71 ± 4	91 ± 4*	89 ± 3*	101 ± 5*
Heart rate (% of t = 0)	94 ± 3	96 ± 4	84 ± 5	80 ± 3*	58 ± 4	50 ± 3
Pressure-rate index (% of t = 0)	68 ± 10	51 ± 8	53 ± 8	64 ± 8	48 ± 6	32 ± 6
Purine overflow (nmol/min.gdwt)	100 ± 11	108 ± 10	124 ± 12	44 ± 5*	58 ± 5*	12 ± 2*

values are mean ± SEM; * indicates a significant change ($p < 0.05$) when compared to control.

increase in purine overflow was still apparent (Table I). The pressure-rate index of the treated groups showed no significant difference when compared to the control group at the end of the reperfusion period. However, when all groups were compared with their pressure-rate index values at the end of the control period a dose-dependent improvement of the pressure-rate index was observed for the treated groups. The first significant improvement occurred at 0.3 μM diltiazem. This effect was parallelled by a dose-dependent effect on the coronary flow (Table I).

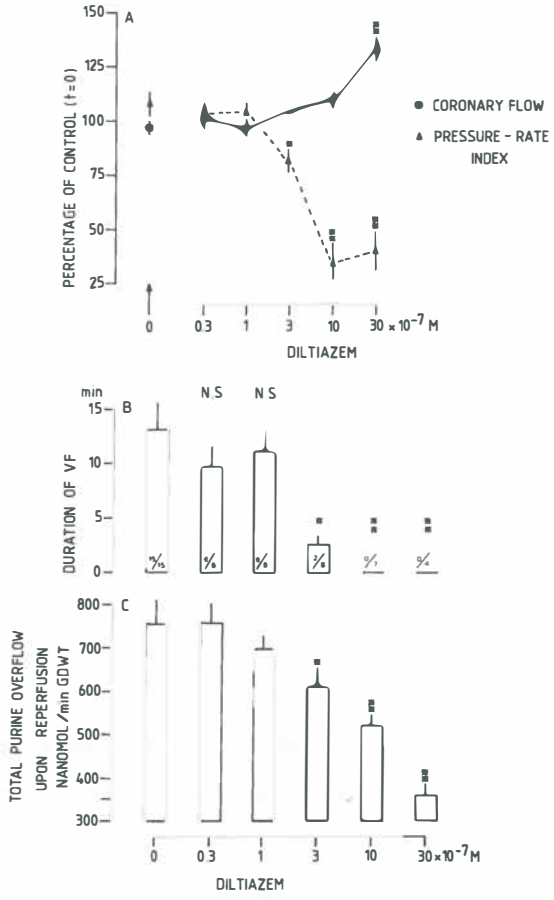

Fig. 1.

A. Effect of different diltiazem concentrations at the end of the control perfusion period (t = 15) on coronary flow (closed circles) and pressure-rate index (closed triangles) are indicated.

B. The incidence and duration (min) of VF upon reperfusion for treated and untreated hearts are indicated. The values in the vertical bars

represent the number of hearts which fibrillated upon reperfusion out of the total number of hearts in each group.

C. The total overflow of adenosine, inosine, hypoxanthine and xanthine during the first min of reperfusion is indicated for the different diltiazem concentrations. The measured values are calculated as nmol per min and expressed per gram dry weight (gdwt).

* $p < 0.05$;**$p < 0.005$ when compared to the untreated group.

Variation in time of diltiazem administration

In this part of the study diltiazem 0.3 μM, i.e. the lowest concentration which reduces the pressure-rate index significantly, was given at different times during the protocol.

Fig. 2 shows the effects of these different treatment schemes on reperfusion induced ventricular fibrillation and purine overflow. The groups which received diltiazem from the control perfusion period onwards and from the ischemic period onwards, showed comparable effects. In contrast, when diltiazem was administered at the beginning of reperfusion no effect on purine overflow during the first min was seen and all these hearts fibrillated, like the untreated hearts. However, the duration of this reperfusion-induced ventricular fibrillation was significantly reduced (Fig. 2). Moreover, diltiazem reduced total purine overflow significantly at 5 min following reperfusion (from 360 $\pm$ 31 for control to 220 $\pm$ 35 nanoM/min.gdwt for treated hearts) and thereafter.

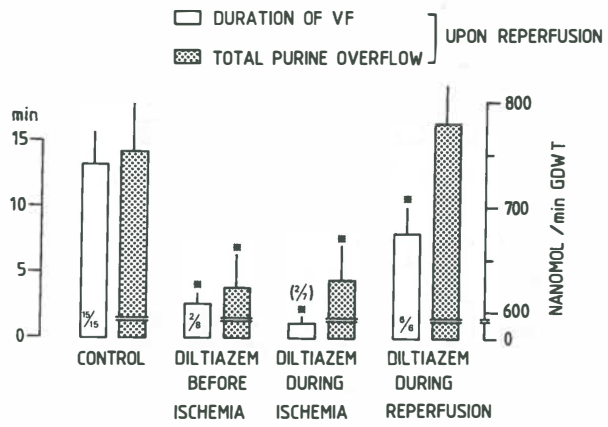

Fig. 2. The duration (min) of VF (open bars) during reperfusion and the total overflow of purines (nanomol/min gdwt: hatched bars) during the first min of reperfusion are indicated for different diltiazem (0.3 μM) treatment protocols. The values in the open vertical bars represent the incidence of VF upon reperfusion.

*$p < 0.05$ when compared to the untreated group

B. Closed chest pig model

The effects of diltiazem were also evaluated in the anesthetized pig using a slightly different experimental procedure. Fig. 3 shows the hemodynamic effects of diltiazem at the end of the control period, the

ischemic period and the reperfusion period. Treatment during the control

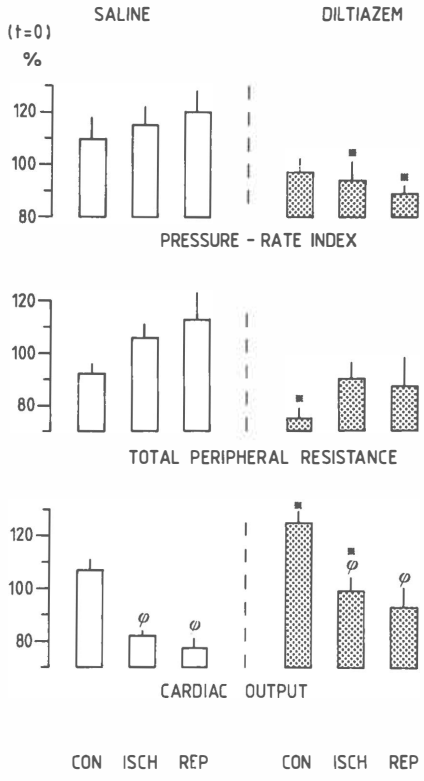

Fig. 3. Changes in hemodynamic parameters are indicated for the untreated (open bars) and the diltiazem group (hatched bars) at the end of the control period (CON), at the end of the ischemic period (ISCH) and after 30 min repefusion (REP).

* $p < 0.05$ when compared to the untreated group in the same period

φ $p < 0.05$ when compared to the values during the control period in the same group

period resulted in a decrease in total peripheral resistance and in an increase in cardiac output but had no significant effect on the pressure-rate index. Oxygen extraction, i.e. the arterial-venous difference in PO from coronary venous and arterial blood, was significantly lower under diltiazem: 7.93 ± 1.0 kPa for the control group versus 5.88 ± 0.9 kPa for the diltiazem group. Occlusion of the LAD by inflating the balloon catheter resulted in a significant reduction of the cardiac output for both untreated and treated pigs when compared to control period values. However, cardiac output of treated hearts remained significantly higher when compared to untreated hearts. At the end of the reperfusion period cardiac output was still impaired in both groups and, although the output of the treated group was still higher, differences were no longer significant. During ischemia and reperfusion the pressure-rate index of the untreated animals showed an increase which was not significant when compared to control period values. In contrast, pressure-rate index of the treated group decreased significantly during both periods compared to controls.

During the ischemic period an increase of the ventricular rate, i.e. the rate independent of the site of origin, was observed for untreated hearts. The ventricular rate of the diltiazem treated group remained unchanged and was significantly lower when compared to the untreated group during the second part of the ischemic period. Upon reperfusion, all untreated animals showed an accelerated idioventricular rhythm which lasted for 22 ± 5 min after which sinus rhythm returned. In contrast, only two treated hearts had for 2 resp 8 min an accelerated

idioventricular rhythm. The mean ventricular rate of the diltiazem group was significantly lower when compared to the untreated group during the whole reperfusion period (Fig. 4).

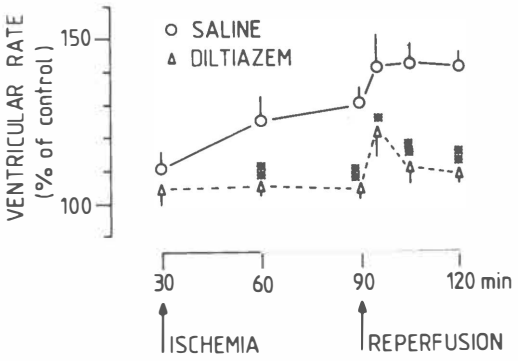

Fig. 4. The rate of ventricular depolarizations independent of the site of origin is indicated for the untreated and the diltiazem group.
* $p < 0.05$; ** $p < 0.005$ when compared to the untreated group

Upon reperfusion of the LAD a substantial rise in noradrenaline levels in the coronary sinus blood of both groups was detected. These noradrenaline levels remained elevated for untreated hearts. However, noradrenaline levels of treated hearts decreased after 5 min and were significantly lower when compared to untreated hearts. Interestingly, adrenaline levels underwent no significant change during ischemia or reperfusion and were comparable in both groups (Fig. 5).

The influence of diltiazem on creatine kinase release wasalso assessed. The maximum creatine kinase level for both groups occurred

after 90 min reperfusion and was 8800 ± 1100 U/L for the untreated group

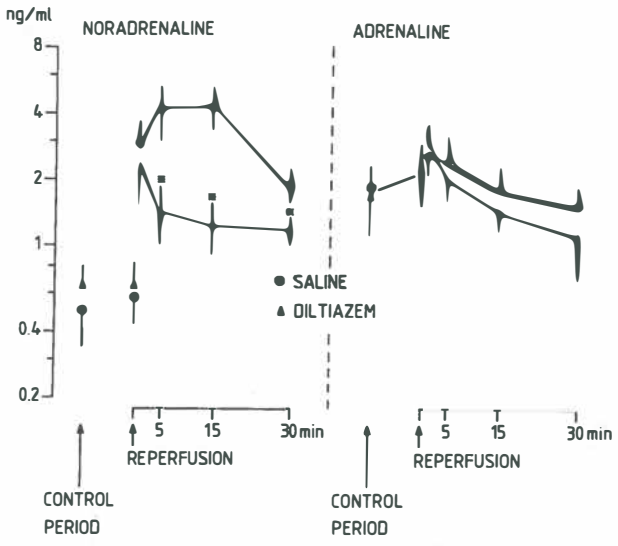

Fig. 5. The concentrations (ng/ml) of noradrenaline (left panel) or adrenaline (right panel) in the coronary sinus blood are indicated for the untreated group (closed circels) and the diltiazem group (closed triangles).

*$p < 0.05$ when compared to the untreated group.

and 4600 ± 700 U/L for the diltiazem group. Diltiazem had no effect on rate constants of creatine kinase kinetics.

After the first experiment all animals recovered within 15 hours. Within 4 days after the first experiment 4 out of 8 untreated animals died suddenly, probably due to severe rhythm disturbances since autopsy

revealed no non-cardiac cause. In contrast, no sudden deaths occurred in the diltiazem group ($p < 0.05$).

In 3 of the 4 surviving untreated pigs sustained ventricular tachycardia could reproduciably be induced with programmed electrical stimulation. However, in none of the diltiazem treated pigs ventricular tachycardia was inducible ($p < 0.05$).

DISCUSSION

In the present study we investigated the effects of diltiazem, a clinically widely used calcium channel blocking agent, in two models of myocardial ischemia and reperfusion. In the isolated rat heart the observed effects are independent of changes in pre- and afterload, which play an important role in the in vivo situation since inhibition of the slow channel results in systemic vasodilation (35). For the in vivo experiments we chose the porcine heart, since it lacks a significant collateral blood flow, and thus occlusion of the LAD results in an homogeneous ischemic region (52).

The duration of the period of ischemia preceding reperfusion was carefully chosen since this is perhaps the most important determinant of the vulnerability of cardiac tissue to reperfusion phenomena (32). Both time periods, 15 min in the isolated rat heart and 60 min in the porcine heart, have been shown to induce a state of reversible injury with the onset of irreversible injury (7,27).

The results obtained in the isolated rat heart show that diltiazem, when present before or at the onset of ischemia, can protect the myocardium against reperfusion-induced arrhythmias in a dose-dependent manner. Both the incidence and duration of ventricular fibrillation were significantly reduced. These effects correlated with the concentration dependent effects of diltiazem on the pressure-rate index during the control period and with the overflow of ATP catabolites during ischemia and reperfusion. The negative chronotropic and inotropic effects of diltiazem in normoxic hearts have also been established by other authors (21,34) and they suggest that the ATP-sparing effect of diltiazem, administered before or at the onset of ischemia, is due to a reduction in oxygen-consumption. However, in agreement with De Jong et al. (22), we found no correlation between myocardial function and purine overflow (Table I) since no dose-dependent reduction of the pressure-rate index was found during ischemia in the isolated heart. In our pig model, diltiazem even improved cardiac performance significantly when compared to control. This has also been shown by other investigators in dogs (43). This suggests that a reduction in myocardial oxygen consumption is not the only protective mechanism of diltiazem. Improvement of oxygen supply is unlikely to occur since no significant collateral flow is present in our models.

When diltiazem was administered upon reperfusion no effects were observed on the incidence of ventricular fibrillation. However, the duration of fibrillation was significantly reduced. Moreover, the overflow of purines after 5 min of reperfusion was also significantly less when compared to the control group. Weishaar and Bing (50) did not

find a protective effect of diltiazem, administered 5 min prior to reperfusion, on myocardial ATP, although intracellular creatine phosphate content increased. In our study late administration of diltiazem had no effect on the washout of purines accumulated during ischemia, but apparently reduced the impairment of ATP metabolism due to reperfusion per se. This finding supports the suggestion that calcium antagonists may reduce the rate of calcium entry in the initial phase of reperfusion although ATP stores are depleted. In this way a further cascade of events, resulting in uncontrolled calcium entry, cell death and tissue necrosis is prevented or at least delayed (39). The same mechanism may in part explain the decreased ATP breakdown observed during ischemia.

In the in vivo model, accelerated idioventricular rhythm upon reperfusion was prevented by diltiazem and coincided with a reduction of the noradrenaline overflow (figs 4 and 5). This observation is in agreement with the findings of Nayler and Sturrock (38) who reported an ischemia-reperfusion induced depletion of endogenous stores of noradrenaline which was prevented by diltiazem. The mechanisms which lead to a depletion of noradrenaline during ischemia and reperfusion are not clear at present and may be indirectly or directly determined. In the first situation, damage of the myocardial cells may lead to either increased extracellular potassium concentrations (3) or the formation of membrane active metabolites such as lysophosphoglycerides (5). Both will induce depolarization of the intracardial sympathetic nerve ending and thus promote noradrenaline overflow. In a direct manner, the nerve ending itself may be subject of damage: the uptake and storage

mechanisms may be impaired as a direct consequence of an insufficient availability of high energy substrates during ischemia (49). A decreased ATP catabolism was observed in our in vitro experiments when hearts were pretreated with diltiazem and thus its effect on noradrenaline overflow may be explained in terms of reuptake and storage preservation. Finally, the protective mechanism of diltiazem on noradrenaline overflow may be the ability of this compound to reduce desintegration of the membrane itself caused by ischemia and reperfusion (39,45) and this protection may extend to the noradrenaline storage vesicles. That diltiazem may reduce cellular damage is apparent in this study from a reduced creatine kinase release.

Interestingly, a recent study indicates that during intracoronary thrombolysis in patients, the presence of an increased idioventricular rhythm is an indicator of the success of recanalization (16). Alpha-adrenoceptor blocking drugs have been shown to be very effective in reducing the incidence of arrhythmias during reperfusion. An enhanced alpha-adrenergic responsiveness (46) and an increased number of alpha-receptors(4) occur during ischemia and reperfusion. Thus, increased local myocardial noradrenergic activity may be crucial in the development of arrhythmias during ischemia and reperfusion. This causal relationship between noradrenaline and reperfusion arrhythmias is supported by the findings in our study. The fact that adrenaline levels underwent no significant change upon reperfusion indicates a localized interaction and not an increase in peripheral adrenal activity.

The lack of sudden death in the first week after ischemia and subsequent reperfusion in the treated group suggests that diltiazem may

also prevent late arrhythmias responsible for the occurrence of sudden death in man. These results are in agreement with the outcome of programmed electrical stimulation one week after the acute experiment. In the treated group none of the animals was inducible whereas three out of four animals in the control group were inducible to sustained ventricular tachycardia. Bigger et al. (2) suggested a relation between the mass of scar tissue and the likeliness of lethal ventricular arrhythmias. This hypothesis is in agreement with our observations that maximal creatine kinase levels after reperfusion in the diltiazem treated group were significantly lower than in the control group, indicating a reduction of early tissue injury and possibly also a reduction of the formation of scar tissue late after ischemia and reperfusion. To our knowledge, this is one of the first reports of a reproducible sudden death model with a relatively high incidence, which needs further investigation.

In conclusion the results of this study show that the calcium antagonist diltiazem can beneficially influence the events during ischemia and reperfusion, both in vitro and in vivo. Only part of this effect can be explained by its effect on myocardial contractility and chronotropy. Probably a multiplicity of mechanisms, all leading to a gain in cellular calcium during ischemia and reperfusion are involved. Of these mechanisms, ATP-depletion and loss of endogenous noradrenaline which are both reduced by diltiazem, may predominate. Furthermore, the severity of ischemia and the extent of injury at the moment of reperfusion will largely determine the effectivity of calcium channel blockade. This complex situation may explain the controversial results

obtained in several experimental studies. Thus, in the even more variable clinical setting, a careful study design will be needed to evaluate the effects of diltiazem and other calcium antagonists in the salvage of myocardial tissue, jeopardized by ischemia and reperfusion.

REFERENCES

1. Axelrod PJ, Verrier RL, Lown B (1975) Vulnerability to ventricular fibrillation during acute coronary arterial occlusion and release. Am. J. Cardiol. 36: 776-782
2. Bigger JT, Dresdale RJ, Heissenbuttel RH, Weld FM, Wit AL. (1977) Ventricular arrhythmias in ischemic heart disease: mechanism, prevalence, significance and management. Prog. Cardiovas. Dis 19: 255-300.
3. Borda L, Schuchleib R, Henry PD (1977) Effects of potassium on isolated canine coronary arteries: modulation of adrenergic responsiveness and release of norepinephrine. Circ Res 14: 778-786
4. Corr PB, Hayman JA, Kramer JB, Kipnis RJ (1981) Increased alpha-adrenergic receptors in ischaemic cat myocardium - a potential mediator of electrophysiological derangements. J. Clin. Invest. 67: 1232-1236
5. Corr PB, Snyder DW, Lee BI, Gross RW, Keim CR, Sobel BE (1982) Pathophysiological concentrations of lysophosphatides and the slow response. Am J Physiol 243: 187-195
6. Corr PB, Witkowski FX (1983) Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischaemic myocardium. Circulation 68, suppl 1: 16-24
7. Crome R, Hearse DJ, Manning A (1983) Relationship between cellular cyclic AMP content and the incidence of ventricular fibrillation upon reperfusion after varying periods of ischaemia. J. Mol. Cell. Cardiol. 15 (suppl 1): 180

8. Elharrar V, Zipes DP (1977) Cardiac electrophysiologic alterations during myocardial ischemia. Am J Physiol 232: H329-H345

9. Ganote CE, Liu SY, Safavi S, Kaltenbach JP (1981) Anoxia, calcium and contracture as mediators of myocardial enzyme release. J. Mol. Cell. Cardiol. 13: 93-106

10. Ganote CE, Seabra-Gomes R, Nayler WG, Jennings RB (1975) Irreversible myocardial injury in anoxic perfused rat hearts. Am. J. Pathol. 80: 419-450

11. Geary GG, Smith GT, Suehiro GT, McNamara JJ (1982) Failure of nifedipine therapy to reduce myocardial infarct size in the baboon. Am. J. Cardiol. 49: 331

12. Gilst WH van, Langen CDJ de (1982) Ischemia-reperfusion induced arrhythmias in the isolated rat heart. Pharm Weekblad Sci ed 45: 160

13. Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRH, Langen CDJ de (1983) Improved functional recovery of the isolated rat heart after 24 hours of hypothermic arrest with a stable prostacyclin analogue (ZK 36 374). J Mol Cell Cardiol 15: 789-792

14. Gilst WH van, Graeff PA de, Kingma JH, Wesseling H, Langen CDJ de (1984) Captopril reduces purine loss and reperfusion arrhythmias in the rat heart after coronary artery occlusion. Eur J Pharmacol 100: 113-117

15. Gobel FL, Nordstrom LA, Nelson RR, Jorgenson CR, Wang Y (1978) The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 57: 549-556

The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 57: 549-556

16. Goldberg S, Greenspon AJ, Urban PL, Muza B, Berger B, Walinsky P, Maroko PR (1983) Reperfusion arrhythmia: A marker of restoration of antegrade flow during intracoronary thrombolysis for acute myocardial infarction. Am Heart J 105: 26

17. Harmsen E, Jong JW de, Serruys PW (1981) Hypoxanthine production by ischemic heart demonstrated by high performance liquid chromatography of blood purine nucleosides and oxypurines. Clin Chim Acta 115: 73

18. Hearse DJ (1983) Critical distinctions in the modification of myocardial cell injury. In: Opie LH (ed) Calcium Antagonists. New York: Raven Press

19. Hearse DJ, Humphrey SM, Bullock GR (1978) The oxygen paradox and the calcium paradox: Two facets of the same problem. J. Mol. Cell. Cardiol. 10: 641-668

20. Jennings RB, Ganote CE, Reimer KA (1975) Ischemic tissue injury. Am. J. Pathol. 81: 179-198

21. Jolly SR, Menahahan LA, Gross GJ (1981) Diltiazem in myocardial recovery from global ischemia and reperfusion. J. Mol. Cell. Cardiol. 13: 359-372

22. Jong JW de, Harmsen E, De Tombe PP (1984) Diltiazem administered before or during myocardial ischemia decreases adenine nucleotide catabolism. J. Mol. Cell. Cardiol. 16: 363-370

24. Kannengieser GJ, Lubbe WF, Opie LH (1975) Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. J Mol Cell Cardiol 7: 135-151

25. Kaplinsky ES, Ogawa S, Michelson EL, Dreifus LS (1981) Instanteneous and delayed ventricular arrhythmias after reperfusion of acutely ischemic myocardium: evidence for multiple mechanisms. Circulation 63: 333-340

26. Karlsberg RP, Henry PD, Ahmed SA, Sobel BE, Roberts R (1977) Lack of protection of ischemic myocardium by verapamil in conscious dogs. Eur. J. Pharmacol. 42: 339

27. Klein HH, Schubothe M., Nebendahl K, Kreuzer H (1984) Temporal and spatial development of myocardial infarcts in porcine hearts without significant collateral blood flow. Texas Heart Inst. J. 11: 154-159

28. Koomen JM, Gilst WH van, Zimmerman ANE, Noordwijk J van (1982) A concentration-dependent biphasic positive inotropic action of ouabain on isolated hearts of rat and guinea-pig. Arch Int Pharmacodyn Ther 255: 2

29. Langen CDJ de, Gilst WH van, Wesseling H (1985) Sustained protection by iloprost of the porcine heart in the acute and chronic phases of myocardial infarction. J. Cardiovasc. Pharmacol (in press)

30. Lowe JE, Reimer KA, Jennings RB (1978) Experimental infarct size as a function of the amount of myocardium at risk. Am. J. Pathol. 90: 363

31. Lubbe WF, Daries PS, Opie LH (1978) Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc Res 12: 212-220

32. Manning AS, Hearse DJ (1984) Reperfusion-induced arrhythmias: mechanisms and prevention. J Mol Cell Cardiol 16: 497-518

33. Murdock DK, Loeb JM, Euler DE, Randall WC (1980) Electrophysiology of coronary reperfusion, a mechanism for reperfusion arrhytymias. Circulation 61: 175-182

34. Nakajima H, Hoshiyama M, Yamashita K, Kiyomoto A (1975) Effect of diltiazem on electrical and mechanical activity of isolated cardiac ventricular muscle of guinea pig. Jpn J. Pharmacol. 25: 383-392

35. Nayler WG (1980) Calcium antagonists. Eur. Heart J. 1: 225-237

36. Nayler WG (1980) Cardioprotective effects of calcium ion antagonists in myocardial ischemia. Clin. Invest. Med. 3: 91-99

37. Nayler WG, Ferrari R, Williams A (1980) Protective effect of pretreatment with verapamil, nifedipine and propranolol on mitochondrial function in the ischemic and reperfused myocardium. Am. J. Cardiol. 46: 242

38. Nayler WG, Sturrock WJ (1983) An inhibitory effect of verapamil and diltiazem on the release of noradrenaline from ischaemic and reperfused hearts. J. Mol. Cell. Cardiol. 16: 331-344

39. Nayler WG, Sturrock WJ, Panagiotopoulos S (1985) Calcium and myocardial ischemia. In: Control and manipulation of calcium movement, ed. JR Parratt, Raven Press New York, 303-324

40. Neely JR, Whitmer KM, Mochizuki S (1976) Effects of mechanical activity and hormones on myocardial glucose and fatty acid utilization. Circ Res 38 (I): 22 - 30.

41. Oliva PB, Breckenridge JC (1977) Arteriographic evidence of coronary arterial apasm in acute myocardial infarction. Circulation 56: 366-374

42. Penkoske PA, Sobel BE, Corr PB (1978) Disparate electrophysiological alterations accompanying dysrhythmia due to coronary occlusion and reperfusion in the cat. Circulation 58: 1023-1035

43. Perez JE, Sobel BE, Henry PD (1980) Improved performance of ischemic canine myocardium in response to nifedipine and diltiazem. Am. J. Physiol. 239: H658-H668

44. Reimer KA, Lowe JE, Jennings RB (1977) Effect of the calcium antagonist verapamil on necrosis following temporary coronary artery occlusion in dogs. Circulation 55: 581

45. Sakai K, Gebhard MM, Spieckermann PG, Bretschneider HJ (1975) Enzyme release resulting from total ischemia and reperfusion in the isolated, perfused guinea pig heart. J. Mol. Cell. Cardiol. 7: 827-840

46. Sheridan DJ, Penkoske PA, Sobel BE, Corr PB (1980) Alpha-adrenergic contributions to dysrhythmia during myocardial ischaemia and reperfusion in cats. J. Clin. Invest. 65: 161-171

47. Stam H, Jong JW de (1977) Sephadex-induced reduction of coronary flow in the isolated rat heart: a model for ischemic heart disease. J Mol Cell Cardiol 9: 633

48. Tzivoni D, Keren A, Granot H, Gottlieb S, Benhorin J, Stern S (1983) Ventricular fibrillation caused by myocardial reperfusion in Prinzmetal's angina. Am Heart J 105: 323-325

49. Wakade AR, Furchgott RF (1968) Metabolic requirements for the uptake and storage of norepinephrine by the isolated left atrium of the guinea-pig. J Pharmacol Exp Ther 163: 123-135

50. Weishaar RE, Bing RJ (1980) The beneficial effect of a calcium channel blocker, diltiazem, on the ischemic-reperfused heart. J. Mol. Cell. Cardiol. 12: 993-1009

51. Westerink BHC (1983) Analysis of trace amounts of catecholamines and related compounds in brain tissue: a study near the detection limit of liquid chromatography with electrochemical detection. J Liquid Chrom 6, 12: 2337-2351

52. White FC, Bloor CM (1981) Coronary collateral circulation in the pig: correlation of collateral flow with coronary bed size. Basic Res. Cardiol. 76: 189

53. Yellon DM, Hearse DJ, Maxwell MP, Chambers DE, Downey JM (1983) Sustained limitation of myocardial necrosis 24 hours after coronary artery occlusion: verapamil infusion in dogs with small myocardial infarcts. Am. J. Cardiol. 51: 1409

APPENDIX VII

VENTRICULAR ARRHYTHMIAS AND PURINE LOSS UPON REPERFUSION OF ISCHEMIC MYOCARDIUM: COMPARISON OF ZK 36 374 AND DILTIAZEM

W.H. van Gilst, J.A. Terpstra and C.D.J. de Langen.

Prostaglandins and other eicosanoids in the cardiovascular system.
Ed. Schrör, Karger, Basel: 207-212, 1985

VENTRICULAR ARRHYTHMIAS AND PURINE LOSS UPON REPERFUSION OF ISCHEMIC MYOCARDIUM: COMPARISON OF ZK 36 374 AND DILTIAZEM

INTRODUCTION

Reperfusion of the ischemic myocardium can lead to a paradoxical extension of the damage due to ischemia alone (1, 2). The biochemical basis for this finding is not fully understood at present but is presumably related to a loss in membrane integrity (3) and the inability of the ischemic myocardium to maintain normal calcium homeostasis upon reperfusion (4, 5, 6). In this regard, calcium antagonists may have a protective effect on ischemic and reperfused myocardium, since a slow channel blockade can prevent a detrimental accumulation of calcium ions in jeopardized myocardial cells (7).

By a different mechanism, prostacyclin and a stable prostacyclin analogue, ZK 36 374 (iloprost), were also demonstrated to preserve myocardial cell integrity during ischemia by inhibition of lysosomal enzyme release (8, 9, 10). These agents may, therefore, also protect myocardial cells by preventing calcium overloading via a different mechanism.

In this study we compared the protective efficacy of a calcium antagonist, diltiazem, and a stable prostacyclin analogue, ZK 36 374, in an isolated rat heart subjected to reversible coronary ligation. Furthermore, we investigated whether their protective effect resulted in

less ischemic damage or was mainly due to the prevention of deleterious reperfusion phenomena.

MATERIAL AND METHODS

The hearts of male Wistar rats (± 300 g) were subjected to Langendorff's non-recirculating perfusion. The perfusion buffer was modified Tyrode's solution (10), and the perfusion pressure was maintained at 60 mmHg. The hearts were beating spontaneously. Acute regional myocardial ischemia was produced by ligation of the left main coronary artery. Reperfusion of the ischemic tissue was achieved by releasing the ligation. After an equilibration period and a control period of 15 min, local ischemia was provoked for 15 min; subsequent reperfusion lasted 30 min. The hearts were subdivided at random into three groups; an untreated group (n=8), a diltiazem (50 nM) group (n=6) and a ZK 36 374 (4 nM) group (n=6). Treatment was continued throughout the whole experiment. The pressure rate index was used to evaluate mechanical function and was calculated as the product of left ventricular pressure and heart rate. Electrophysiologic function was monitored by continuous registration of the ECG. Overflow of adenosine and its catabolites was used as a sensitive indicator of nucleotide breakdown and, thus, cellular damage (10). The data are expressed as the means ± SEM. Probability values were calculated by Student's t-test, and p-values less than 0.05 were considered to be significant.

RESULTS

The effects of diltiazem and ZK 36 374 on coronary flow, pressure rate index and total purine overflow at the end of the preocclusion period are depicted in table I.

Table I. Effects of ZK 36374 and diltiazem on mechanical function and total purine overflow

		End preocclusion period	End occlusion period	End reperfusion period
Untreated	CF	97 ± 2	56 ± 4	87 ± 2
	PR	105 ± 4	32 ± 4	58 ± 6
	PO	7.5 ± 0.5	67 ± 5	20 ± 4
ZK 36 374 (8 nM)	CF	100 ± 3	63 ± 4	97 ± 2*
	PR	102 ± 6	41 ± 5	92 ± 12*
	PO	8.5 ± 1	55 ± 9	10 ± 3*
Diltiazem	CF	115 ± 2*	63 ± 6	107 ± 4*
	PR	68 ± 7*	34 ± 3	85 ± 7*
	PO	6.5 ± 1	62 ± 9	8 ± 1*

CF = coronary flow (% of control); PR = pressure rate index (% of control);
PO = purine overflow (nmol/min. g dwt)
* indicates a significant ($p < 0.05$) change when compared to untreated hearts

The pressure rate index and coronary flow were significantly altered by diltiazem treatment; ZK 36 374 treatment had no significant effects during this period. The recovery of the mechanical function of treated hearts after 30 min of reperfusion was significantly better than the recovery of untreated hearts (table I).

Upon reperfusion, the inosine overflow was significantly less in the ZK 36 374-treated hearts (fig. 1). ZK 36 374 treatment did not affect the adenosine overflow during reperfusion (fig. 2). In marked

contrast, diltiazem reduced the adenosine overflow to hardly detectable

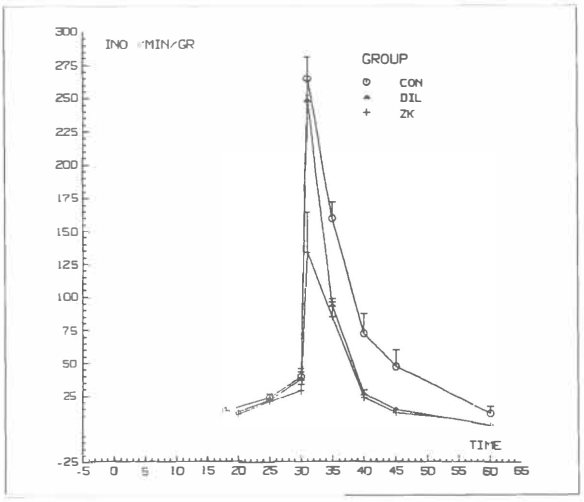

Fig. 1. Time-course of the overflow of inosine during the ischemic and reperfusion periods for the untreated hearts (CON), diltiazem (DIL)- and ZK 36 374 (ZK)-treated hearts. The measured values are calculated as nmol/min, expressed per g dry weight and plotted against time on a semilogarithmic scale. Symbols represent means ± SEM.

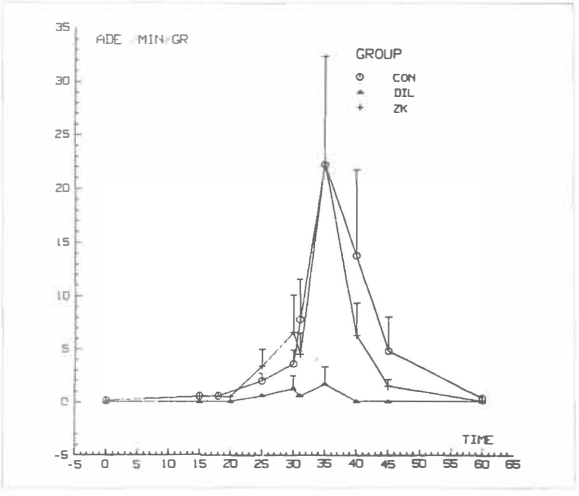

Fig. 2. Time-course of the overflow of adenosine during the ischemic and reperfusion periods für for the untreated hearts (CON) resp. diltiazem (DIL)- and ZK 36 374 (ZK)-treated hearts. The measured values are calculated as nmol/min, expressed per g dry weight and plotted against time on a semilogarithmic scale. Symbols represent means ± SEM.

levels (fig. 2). The overflow of inosine upon reperfusion of diltiazem-treated hearts was not significantly different from the untreated group (fig. 1). Upon reperfusion, all untreated hearts showed ventricular fibrillation for a period of 16 ± 3.7 min. However, ventricular fibrillation was observed in only three ZK 36 374-treated hearts for a period of 7.3 ± 3.2 min. Four of the diltiazem-treated hearts showed ventricular fibrillation upon reperfusion for a period of 8.2 ± 2.0 min (table II).

Table II. Incidence and duration of reperfusion arrhythmias after 15 min ischemia

	Incidence of VF	Duration of VF (min)
Control	8/8	16 ± 3.7
ZK 36374	3/6	7.3 ± 3.2 $p < 0.05$
Diltiazem	4/6	8.2 ± 2.0 $p < 0.05$

DISCUSSION

This study demonstrates that both a stable prostacyclin analogue (ZK 36 374) and a calcium antagonist (diltiazem) improve mechanical and electrophysiologic recovery upon reperfusion after 15 min of normothermic ischemia.

Differences in the mechanisms of action between the drugs were apparent from the purine overflow data. During the first minute of

reperfusion, a massive overflow of inosine was detected in the coronary effluent of untreated hearts. Adenosine overflow was hardly detectable at this moment. Presumably, the adenosine leaked from the ischemic cells is converted to inosine during the ischemic period, i.e., the high rate of adenosine deamination (11) to inosine is probably related to this lack in adenosine overflow. In subsequent samples, a delayed adenosine overflow was observed in untreated hearts. Presumably, this delayed adenosine overflow indicates an additional damage upon reperfusion in this model.

The prostacyclin analogue (ZK 36 374) exerts its main effect on the initial washout of adenosine catabolites. It is proposed that this agent protects mainly during the ischemic period, probably by preserving membrane integrity (8, 9). However, this prostacyclin analogue lacks protective effects during reperfusion. It cannot prevent the elevated ATP catabolism, reflected in the delayed overflow of adenosine, which may be due to calcium overloading (4, 5, 6, 7) of surviving cells jeopardized by ischemia.

In contrast, diltiazem had no significant effect on inosine overflow during the first minute of reperfusion. However, diltiazem showed ATP-sparing properties during reperfusion, reflected in an abolishment of the delayed adenosine overflow. Whether this effect is caused by an enhanced adenosine reuptake or by a decrease in ATP catabolism by preventing the calcium overload of surviving jeopardized myocardial cells is subject to further study.

REFERENCES

1. Reimer KA, Hill ML, Jennings RB. Prolonged depletion of ATP and of the adenine nucleotides folllowing reversible myocardial ischemic injury in dogs. J Mol Cell Cardiol 13, 229-239, 1981.
2. Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 66, 1146-1149, 1982.
3. Sobel BE, Corr PB, Robison AK, Goldstein RA, Witkowski FX, Klein MS. Accumulation of lysophosphoglycerides with arrhythmogenic properties in ischemic myocardium. J Clin Invest 62, 546-553, 1978.
4. Hearse DJ. Reperfusion of the ischemic myocardium. J Mol Cell Cardiol 9, 605-616, 1977.
5. Nayler WG. The role of calcium in the ischemic myocardium. Am J Path 102, 262-270, 1981.
6. Murphy ML, Peng CF, Kane JJ, Straub KD. Ventricular performance and biochemical alteration of regional ischemic myocardium after reperfusion in the pig. Am J Cardiol 50, 821-828, 1982
7. Henry PD, Schuchleib R, Davis J, Weiss ES, Sobel BE. Myocardial contracture and accumulation of mitochondrial calcium in ischemic rabbit heart. Am J Physiol 233, 677-684, 1977.
8. Ogletree ML, Lefer AM, Smith JB, Nicolaou KC. Studies on the protective effect of prostacyclin in acute myocardial ischemia. Eur J Pharmacol 56, 95-103, 1979.

9. Schrör K, Ohlendorf R, Darius H. Beneficial effects of a new carbacyclin derivative, ZK 36 374, in acute myocardial ischemia. J Pharmac Exp Ther 219, 243-249, 1981.

10. Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRH, Langen CDJ de. Improved functional recovery of the isolated rat heart after 24 hours of hypothermic arrest with a stable prostacyclin analogue (ZK 36 374). J Mol Cell Cardiol 15, 789-792, 1983.

11. Berne RM, Rubio R. Adenine nucleotide metabolism in the heart. Circ Res 34 and 35, suppl. 3, 109-120, 1974.

APPENDIX VIII

REPRODUCIBLE POST EXERCISE VENTRICULAR TACHYCARDIAS: A CASE OF REPERFUSION ARRHYTHMIAS?

W.H. van Gilst, J.H. Kingma and K.I. Lie

Submitted for publication in the
American Journal of Cardiology

REPRODUCIBLE POST EXERCISE VENTRICULAR TACHYCARDIAS: A CASE OF REPERFUSION ARRHYTHMIAS?

The experimental findings that malignant ventricular arrhythmias not only occur immediately following coronary artery occlusion but also during subsequent reperfusion is well documented nowadays (1). In clinical practice, however, there are only few reports confirming the occurrence of these reperfusion arrhythmias in man. Most of these reports described patients with variant angina who developed ventricular arrhythmias immediately following the termination of ischemia, when the ST segment had returned to normal (2,3). However, several authors have suggested that abnormal coronary vasomotion may play an important role in many forms of myocardial ischemia and also in exercise-related angina (4). We describe here a patient who developed reproducible ventricular tachycardias during rest, several minutes after graded exercise testing (GXT) by upright bicycle ergometry. The role of reperfusion as a possible cause of this type of ventricular tachycardia is discussed.

A 53-year-old farmer was referred to our hospital because of dizziness and palpitations occurring upon exercise. There were no other cardiovascular complaints, especially no symptoms of angina pectoris or dyspnoea, on exercise or at rest. The previous history did not mention any other serious medical disease. Physical examination and routine laboratory investigations as well as chest X-ray and ECG did not reveal any abnormalities. Initially the patient was empirically treated with disopyramide phosphate (250 mg b.i.d.). However, he still complained of

palpitations associated with exercise. Therefore, GXT was performed

Figure 1.

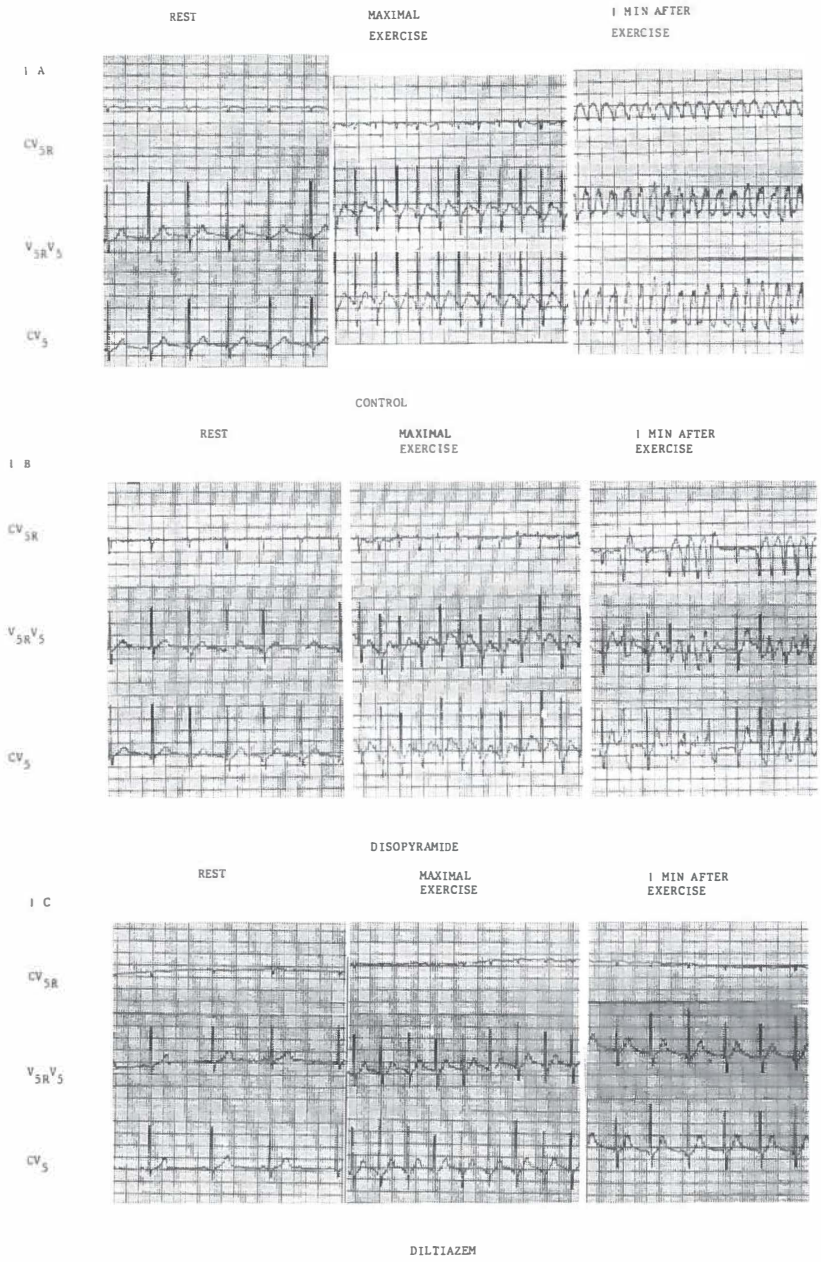

Fig. 1. ECG at rest, maximal exercise and 1 minute after exercise. There are no arrhythmias at maximal exercise. These appear one minute after discontinuation of exercise, which is prevented by diltiazem, but not by disopyramide.

during disopyramide treatment and one week after discontinuation of this drug, in order to induce arrhythmias and investigate the presence of ischemia.

During disopyramide treatment the patient reached a maximum exercise capacity of 220 Watts (W) and a maximal heart rate of 180 bpm. No arrhythmias were observed during exercise, but during the first 10 minutes after exercise incessant, but nonsustained polymorph ventricular tachycardias occurred with a maximal duriation of 1.6 s and a rate of 270 bpm (Fig. 1b). When GXT was repeated after discontinuation of disopyramide the patient reached a maximal exercise capacity of 220 W at 180 bpm. Sustained monomorph ventricular tachycardias appeared one minute after exercise, lasting for 8 minutes (Fig. 1a). During exercise the patient was completely free of arrhythmias. Finally the patient was treated during one week with a calcium antagonist (diltiazem 60 mg q.i.d.). GXT was repeated on the 7th day. The patient reached a maximum exercise capacity of 220 W and maximal heart rate of 150 bpm. No arrhythmias were seen upon exercise, indicating a complete abolishment of the ventricular tachycardia by diltiazem. In neither of the three GXT's ST-depression, indicating serious myocardial ischemia was

observed. However, this does not exclude the presence of regional myocardial ischemia.

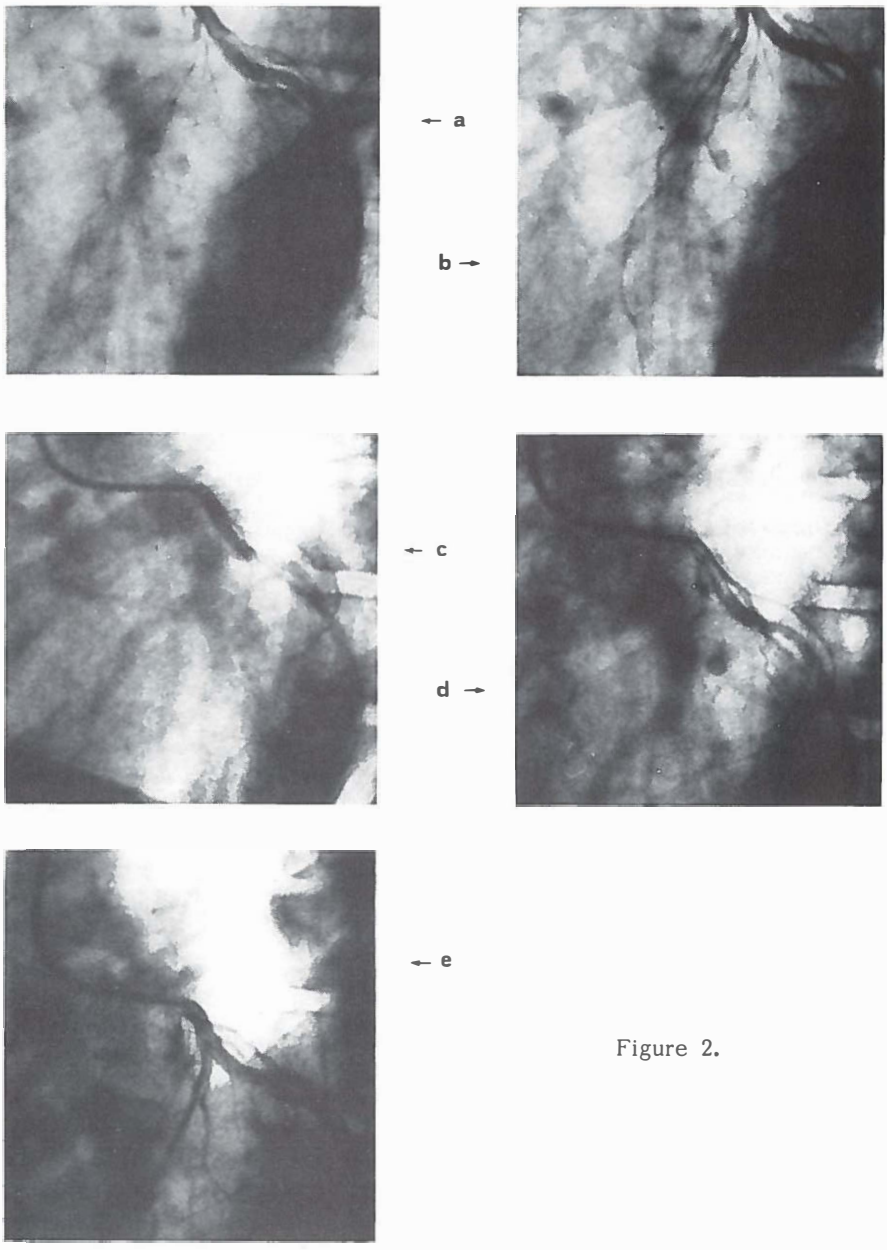

Figure 2.

Fig. 2. Coronary angiogram (left oblique view) showing the left anterior descending branch in the middle and the circumflex system right lateral. Photograph 2a and 2b show the appearance of the LAD narrowed in systole and with larger lumen in diastole. Photograph 2c and 2d show disappearance of the LAD during systole and severe impairment of flow during diastole. Photograph 2e shows "reperfusion" after discontinuation of exercise.

Coronary angiograms using the Sones technique (CAG) were obtained at rest, as well as during and after graded exercise testing by supine bicycle ergometry. Diltiazem treatment was discontinued 24 hours before catheterisation. CAG at control showed no atherosclerotic lesions. However, the left anterior descending branch (LAD) showed a small caliber, ending before the ventricular apex. The appearance of the middle part of the LAD was suggestive of myocardial bridging showing pronounced systolic narrowing (fig. 2a, b). During maximal supine exercise (130 W) contrast injections showed complete occlusion of the LAD in systole (fig. 2c) and subtotal occlusion in diastole (fig. 2d). A contrast injection made within 2 minutes after exercise showed restoration and even an increase of flow of the previously nearly occluded LAD (Fig.2e), associated with repetitive episodes of nonsustained monomorph ventricular tachycardia.

In this case history a patient is described with ventricular tachycardias solely appearing after exercise. Suppression of this ventricular tachycardia was not possible by a class I antiarrhythmic drug, however, these arrhythmias could be completely abolished by diltiazem. Calcium antagonists are usually not very effective in ventricular tachycardias (5). Coronary angiography revealed myocardial bridging of the middle part of the LAD resulting in nearly complete occlusion, even in diastole during exercise. This was followed by restoration and even an increase of flow and associated with the occurrence of ventricular tachycardias. These arrhythmias could be explained as the consequence of reperfusion of a previously ischemic myocardial region. It has been shown that Class I drugs, like disopyramide, are ineffective in suppressing this type of arrhythmias (1). In contrast, calcium antagonists including diltiazem, have been shown to prevent reperfusion damage and to reduce reperfusion-induced arrhythmias (6). This is in accordance with the findings in this case report and supports the suggestion that reperfusion is the trigger for the arrhythmias in this patient. Although the incidence of myocardial bridging seems to be relatively rare, this case report provides evidence that these patients are at risk for life-threatening arrhythmias. Since the incidence of reperfusion arrhythmias depends on the duration of ischemia, it seems imperative to avoid excessive exercise in these patients. Treatment of reperfusion arrhythmias with conventional antiarrhythmic drugs may fail, whereas calcium antagonist can be used successfully.

REFERENCES

1. Manning AS, Hearse DJ (1984) Reperfusion-induced arrhythmias: mechanisms and prevention. J Mol Cell Cardiol 16: 545-548

2. Tzivioni D, Keren A, Granot H, Gottlieb S, Benhorin J, Stern S (1983) Ventricular fibrillation caused by myocardial reperfusion in Prinzmetal's angina. Am Heart J 105: 323-325

3. Previtali M, Klersy C, Salerno JA, Chimienti M, Panciroli C, Marangoni E, Specchia G, Comolli M, Bobba P (1983) Ventricular tachyarrhythmias in Prinzmetal's variant angina: Clinical significance and relation to the degree and time course of S-T segment elevation. Am J Cardiol 52: 19-25

4. Waters DD, Chaitman BR, Dupras G, Theroux P, Mizgala HF (1979) Coronary artery spasm during exercise in patients with variant angina. Circulation 52: 580-585

5. Henry PD (1980) Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. Am J Cardiol 46: 1047

6. Weishaar RE, Bing RJ (1980) The beneficial effect of a calcium channel blocker, diltiazem, on the ischemic-reperfused heart. J Mol Cell Cardiol 12: 993-1009

BIBLIOGRAPHY

Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRH, Langen CDJ de (1983) Improved functional recovery of the isolated rat heart after 24 hours of hypothermic arrest with a stable prostacyclin analogue (ZK 36 374). J Mol Cell Cardiol 15: 789-792.

Gilst WH van, Graeff PA de, Kingma JH, Wesseling H, Langen CDJ de (1984) Captopril reduces purine loss and reperfusion arrhythmias in the rat heart after coronary occlusion. Eur J Pharm 100: 113-117.

Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRH, Langen CDJ de (1985) Improved recovery of cardiac function after 24 h of hypothermic arrest in the isolated rat heart: Comparison of a prostacyclin analogue (ZK 36 374) and a calcium entry blocker (Diltiazam). J Cardiovasc Pharm 7: 520-524.

Gilst WH van, Terpstra JA, Langen CDJ de (1985) Ventricular arrhythmias and purine loss upon reperfusion of ischemic myocardium: Comparison of ZK 36 374 and diltiazem. In: Prostaglandins and other eicosanoids in the cardiovascular system. Ed. K. Schrör, Karger, Basel.

Gilst WH van, Graeff PA de, Wesseling H, Langen CDJ de (1986) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors. A comparison of captopril, enalapril and HOE 498. J Cardiovasc Pharm (in press).

Gilst WH van, Koomen JM (1985) Calcium repletion induced arrhythmias after short periods of calcium-free perfusion in the isolated rat heart. Basic Res Cardiol (submitted).

Gilst WH van, Graeff PA de, Kingma JH, Langen CDJ de, Wesseling H (1986) Effects of diltiazem on ischemia- and reperfusion induced myocardial injury both in vitro and in vivo. J Mol Cell Cardiol (submitted).

Gilst WH van, Kingma JH, Lie KI (1986) Reproducible post exercise ventricular tachycardias: a case of reperfusion arrhythmias? Am J Cardiol (submitted).

Langen CDJ de, Gilst WH van, Wesseling H (1985) Sustained protecton by iloprost of the porcine heart in the acute and chronic phases of myocardial infarction. J Cardiovasc Pharm 7: 924-928

Koomen JM, Gilst WH van, Zimmerman ANE, Noordwijk J van (1982) A concentration-dependent biphasic positive inotropic action of ouabain on isolated hearts of rat and guinea pig. Arch Int Pharmacodyn Ther 255: 2.

Koomen JM, Gilst WH van, Schevers JAM, Wilting J (1984) Biphasic positive inotropic actions of ouabain on rat, guinea-pig and cat heart: a mathematical description. Basic Res Cardiol 79: 102-109.

Graeff PA de, Gilst WH van, Langen CDJ de, Wesseling H (1986) Concentration dependent protection by captopril against ischemia-reperfusion injury in the isolated rat heart. Arch Int Pharmacodyn Ther (in press).

Viersma JW, Berg MP van de, Gilst WH van, Lie KI (1986) Intrinsic sympathomimetic activity of pindolol in patients with sinus node dysfunction. Eur Heart J (in press)

Kingma JH, Graeff PA de, Gilst WH van, Binsbergen E van, Langen CDJ de, Wesseling H (1986) Effects of intravenous captopril on inducible sustained ventricular tachycardia one week after experimental infarction in the anaesthetized pig. J Postgrad Med (in press)

Abstracts

Gilst WH van, Langen CDJ de (1982) Ischemia-reperfusion induced arrhythmias in the isolated rat heart. Pharm Weekblad Sci ed 45: 160.

Gilst WH van, Terpstra JA (1983) Prostacyclin analogue improves recovery of the isolated rat heart after 24 hours of hypothermic cardiac arrest. Circulation 68: III-183.

Gilst WH van, Wierenga BJ, Langen CDJ de (1983) Ischemia and reperfusion induced ventricular arrhythmias; interaction of verapamil and catecholamine. Proc. 24th Dutch Fed. Meeting. 117.

Gilst WH van, Boonstra PW, Terpstra JA, Wildevuur ChRH, Langen CDJ de (1984) Effect of four different calcium antagonists on recovery of the isolated rat heart after 24 h of hypothermic cardiac arrest. Eur Heart J 5 (suppl 1): 143.

Gilst WH van, Daemen BJC, Langen CDJ de (1984) Ischemia and reperfusion induced ventricular arrhytmias; role of calcium and magnesium. Proc 25th Dutch Federation meeting, 316.

Gilst WH van, Houwertjes MC, Wesseling H (1984) Reperfusion arrhythmias in the monkey heart after coronary occlusion. Proc. 9th IUPHAR congress, MacMillan, London, 470.

Gilst WH van, Scaf AHJ (1985) ATP-catabolites as sensitive markers of ischemia- and reperfusion-induced myocardial damage: Use of overflow kinetics to discriminate between these phenomena. N-S Archiv Pharmacol (in press).

Graeff PA de, Gilst WH van, Wesseling H, Langen CDJ de (1983) Effects of captopril on myocardial function during ischemia-reperfusion in the isolated rat heart. Pharm Weekblad Sci ed 5: 264.

Graeff PA de, Gilst WH van, Kingma JH, Langen CDJ de, Wesseling H (1985) Effects of captopril in a closed-chest pig model against ischemia-reperfusion injury. N-S Archiv Pharmacol (in press).

Graeff PA de, Gilst WH van, Langen CDJ de, Wesseling H (1984) Concentration-dependent protection by captopril against reperfusion injury in the isolated rat heart. Circulation 70: II89.

Langen CDJ de, Gilst WH van, Wesseling H (1984) Sustained protection by iloprost of the porcine heart in the acute and chronic phases of myocardial infarction. Circulation 70: II-86

Kingma JH, Wijk LM van, Dunselman PHJM, Gilst WH van, Lie KI, Wesseling H (1984) Pharmacokinetics of flecainide in patients with ventricular arrhythmias and congestive heart failure. Proc. 9th IUPHAR congress, MacMillan, London 477.

Samenvatting

Ruim 50 jaar geleden werd reeds waargenomen dat een plotseling herstel van de antegrade bloedstroom in het ischemische myocard kan leiden tot ernstige, levensbedreigende ritmestoornissen. Pas de laatste tien jaar is de betekenis van deze waarneming volledig duidelijk geworden toen ook bij de mens, dankzij de verdere verbetering van de angiografie, reperfusie aangetoond kon worden.

Het is tegenwoordig algemeen aanvaard dat reperfusie fenomenen een belangrijke rol spelen bij ischemische hartziekten. Vermoedelijk zijn de ritmestoornissen ten gevolge van reperfusie een van de voornaamste oorzaken van plotselinge hartdood bij de mens. Het doel van de experimenten, die in dit proefschrift zijn beschreven, was een bijdrage te leveren aan het ophelderen van het mechanisme van deze ritmestoornissen en te onderzoeken op welke wijze deze ritmestoornissen door farmaca beinvloed kunnen worden.

In Hoofdstuk I wordt een kort overzicht gegeven van de huidige inzichten in de electrofysiologie van reperfusie-aritmieën. Evenals ritmestoornissen ten gevolge van ischemie kunnen reperfusie-aritmieën ingedeeld worden in verschillende categorieen. Zij ontstaan op verschillende tijdstippen na reperfusie en zijn het gevolg van verschillende mechanismen. Het type dat in de eerste seconden na reperfusie ontstaat en waarbij vaak ventrikelfibrilleren optreedt, is vermoedelijk het gevolg van reentry. Het tweede type ritmestoornissen, dat enige minuten na reperfusie optreedt en een duidelijk lagere

incidentie van ventrikelfibrilleren heeft, is vermoedelijk het gevolg van een toegenomen automaticiteit. Zowel ritmestoornissen tijdens ischemie als tijdens reperfusie zijn het gevolg van metabole veranderingen op cellulair niveau in het bedreigde gebied. Het belangrijkste verschil tussen deze twee fenomenen is wel de snelheid waarmee deze veranderingen verlopen.

Het onderzoek naar reperfusie-aritmieën bij patienten wordt vaak bemoeilijkt doordat het exacte moment van reperfusie niet is vast te stellen. In diermodellen is dit probleem afwezig en tevens kunnen zowel de duur als de ernst van de ischemie nauwkeurig gecontroleerd worden. In Hoofdstuk II worden de diermodellen, die in ons onderzoek gebruikt zijn, beschreven. De modellen lopen uiteen van het geisoleerde rattehart tot een in vivo model in de aap. De voor- en nadelen van elk model worden in dit hoofdstuk besproken.

In Hoofdstuk III worden de metabole veranderingen in het ischemische en vervolgens gereperfundeerde gebied en hun relatie met reperfusie-aritmieën beschreven. Aangetoond wordt dat het zogenaamde proces van "calcium overloading" op het moment van reperfusie betrokken is bij het ontstaan van reperfusie-aritmieën. Tevens wordt aannemelijk gemaakt dat catecholaminen, prostaglandines en vrije radicalen betrokken zijn bij het induceren van reperfusie-aritmieën. Het mechanisme en de exacte plaats van deze factoren in de cascade van gebeurtenissen, die plaatsvindt bij reperfusie, is op dit moment nog niet duidelijk.

Tenslotte wordt in dit hoofdstuk ook de afbraak van ATP en het verschijnen van ATP metabolieten in het coronair-veneuze systeem besproken. Door de bepaling van deze metabolieten kan continue

informatie over het ATP metabolisme tijdens ischemie en reperfusie verkregen worden. Door gebruik te maken van de overflow kinetiek kan het effect van beide fenomenen afzonderlijk gekwantificeerd worden. De cellulaire ATP inhoud is een belangrijke maat voor de celfunktie en er is een duidelijke relatie tussen ATP afbraak en het optreden van reperfusie-aritmieën.

In Hoofdstuk IV worden de mogelijkheden om reperfusie-aritmieën farmacologisch te beinvloeden besproken. Drie groepen farmaca, namelijk: calcium antagonisten, prostacycline analoga en angiotensine converting enzym remmers, werden in onze verschillende reperfusie modellen onderzocht. De reductie van reperfusie-aritmieën door de calcium antagonist, diltiazem, kon grotendeels verklaard worden door een ATP sparend effect tijdens ischemie. Echter, wanneer diltiazem pas tijdens reperfusie toegediend werd, was er nog steeds een gering, maar significant effect op reperfusie-aritmieën aanwezig. Hieruit blijkt dat diltiazem ook effect op celschade ten gevolge van reperfusie heeft.

De stabiele prostacycline analoog, iloprost, had voornamelijk effect op de celschade ten gevolge van ischemie, vermoedelijk door preservatie van de membraan integriteit.

De converting enzyme remmers, captopril en HOE 498, bleken in staat om reperfusie-aritmieën effectief te reduceren. De effecten van captopril gingen gepaard met een verdwijnen van de noradrenaline overflow op het moment van reperfusie. Wanneer tegelijkertijd indomethacine werd toegediend trad er een vermindering van de effecten van captopril op. Gepostuleerd wordt dat de gunstige effecten van

captopril een gevolg zijn van de beinvloeding van het prostaglandine metabolisme.

In Hoofdstuk V wordt een overzicht gegeven van de beschikbare data over reperfusie-aritmieën in de klinische situatie. Doordat er geen eenvoudige methoden zijn om reperfusie in patienten vast te stellen en door het onvoorspelbare karakter van reperfusie na ischemie, zijn het aantal gegevens beperkt. Deze problemen zullen eerst opgelost moeten worden alvorens de effectiviteit van farmaca bij reperfusie-aritmieën in patienten geevalueerd kan worden.

DANKWOORD

Dit proefschrift is met de hulp en inzet van velen tot stand gekomen. Iedereen, die op welke wijze dan ook aan het beschreven onderzoek heeft bijgedragen, wil ik heel hartelijk danken. Een aantal van hen noem ik graag met name:

In de eerste plaats mijn promotores: Prof.Dr. H. Wesseling, voor de plezierige manier waarop hij mij heeft begeleid en voor het vertrouwen dat hij steeds in mij heeft gesteld; Prof.Dr. K.I. Lie, die mij de mogelijkheid bood om op de afdeling Cardiologie reperfusiefenomenen bij patienten te onderzoeken; I want to thank, Prof.Dr. W.G. Nayler, for her interest in my work, her expert advises and her offer to travel 20000 kilometers to attend the defence of this thesis.

Cees de Langen en Herre Kingma, voor het enthousiasme waarmee zij hun visie op dit onderzoek op mij overbrachten. Visies, die beide een eigen invalshoek hadden en daardoor synergetisch werkten.

Zonder de assistentie van Winy Wierenga, Katja Wolters-Keulemans, Klaas Bel en Egbert Scholtens zou dit onderzoek zeker onmogelijk zijn geweest. Dank voor het bepalen van grote hoeveelheden monsters, het opereren van vele biggen en het isoleren van nog meer ratteharten en voor alles wat daar bij hoorde.

Pieter de Graeff, onze gelijkgerichte belangstelling deed niet alleen de samenwerking uitstekend verlopen, maar maakte ook menig gezamenlijke congresreis tot een aangename happening. Dank daarvoor.

Arnold Dijk en Willem van de Velde, voor de bereidheid om, desnoods via onorthodoxe wegen, te helpen bij de data opslag en verwerking.

De bijvakstudenten, Peter Nijland, Berry Daemen en in het bijzonder Hans Terpstra, die mij een enkele keer met squash liet winnen.

Jos Koomen, die mijn eerste schreden op het farmacologische pad begeleidde.

Prof.Dr. Ch.R.H. Wildevuur en Piet Boonstra, voor de prettige samenwerking in de hartpreservatie-projekten.

Jacob Pleiter, die altijd verbazend snel perfecte tekeningen, foto's en dia's afleverde.

Ardy Kuperus, voor de plezierige en zorgvuldige manier waarop ze dit proefschrift heeft getypt en gereed gemaakt voor de drukker.